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FERRITIN CRYSTALS IN THE GUT CAECA OF *STEGOCEPHALOIDES CHRISTIANIENSIS* BOECK AND OTHER STEGOCEPHALIDAE (AMPHIPODA: GAMMARIDEA): A FUNCTIONAL INTERPRETATION

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[Plates 1–6]

CONTENTS

Iron-rich octahedral crystals have been described by the senior author in the gut caeca cells of the amphipod *Stegocephaloides christianiensis*. The present investigation revealed their presence in other species in the family Stegocephalidae (*Bathystegocephalus inflatus*, *Euandania ingens*, *Parandania boecki*, *Stegocephaloides auratus*, *S. vanhoffeni*, *Stegocephalus inflatus*, *Phippsiella* spp. and *Parandaniexis* sp. (cf. *mirabilis*). Crystals were not found in *Andaniopsis nordlandica*, *Tetradeion crassum* or *Andaniexis abyssi*, although the latter gave a tissue reaction for iron. Fe cells contain only a single crystal each in all species and crystals consistently increased in size proximally in each caecum. The most distal region of the caecum was devoid of crystals.

Detailed work was confined to *Stegocephaloides christianiensis*, *Stegocephalus inflatus* and

Parandaniexis sp. (cf. *mirabilis*). Caecum ultrastructure of *S. christianiensis* is described: two cell facies (R/F and B cells) are distinguishable. R/F cells (= Fe cells) are columnar, with a terminal brush-border of long microvilli. Lipid globules, glycogen, Fe crystals and Ca granules are found in these cells. B-cells have a luminal border of short, stubby microvilli with an apical complex of membrane-bound vesicles of varying degrees of coalescence. The composition of the Fe crystals has been described using X-ray microprobe analysis. Strong Fe peaks were revealed together with minor peaks for Si, P, S, Cl, K, Ca, Cu and Zn. These elements were identified in the surrounding cytoplasm also. Crystal composition is homogeneous with no separate core. The crystal consists of hexagonally arranged, electron dense cores of 5.8 ± 0.3 nm diameter at intercore distances of 10.5 ± 0.5 nm, 7.5 ± 0.5 nm and 9.5 ± 0.5 nm. Wide angle electron diffraction analysis of the cores gave four rings with *d* spacings of 0.250, 0.223, 0.191 and 0.145 nm (all ± 0.003 nm). On these bases the substance of the crystals is identified as ferritin.

Ferritin crystals are voided in the faeces of *Stegocephaloides christianiensis*, suggesting a role in iron excretion, perhaps as part of a body content regulation process. The content of iron in *S. christianiensis* and a variety of other inshore Amphipoda has been investigated using atomic absorption spectrophotometry. Iron content was non-linearly related to body dry mass in *S. christianiensis* and cannot simply be explained as a consequence of surface adsorption. Iron levels in *S. christianiensis* were higher than in many other species investigated.

The morphology of the mouthparts of *S. christianiensis* has been investigated using scanning electron microscopy. Analyses of fresh stomach contents revealed cnidarian nematocysts which corresponded in size and form with those from *Adamsia carciniopados*, *Pennatula phosphorea* and *Hydractinia echinata*. Behavioural observations on live *S. christianiensis* suggested that *Pennatula* was a likely prey item. Investigations of a range of Cnidaria and of a few known predators of cnidarians (*Pycnogonum*, *Hyperia*) confirmed that the discharged acontia of *Adamsia* and the soft tissues of *Pennatula* contained unusually high concentrations of iron. It is proposed that the production and expulsion of ferritin crystals by *S. christianiensis* and other cnidarian-consuming species in the family Stegocephalidae represents an iron regulation system in animals experiencing a dietary iron challenge.

1. Introduction

This investigation followed from the discovery by one of us (Moore 1979) of iron-rich crystals in the gut caeca of *Stegocephaloides christianiensis* Boeck (figure 3). Since then, we have been able to examine museum material from a range of stegocephalid genera kindly made available by colleagues worldwide (see Acknowledgements). These studies have shown that such crystals are of widespread (though not universal) occurrence within the family Stegocephalidae. We have subjected the crystals of *S. christianiensis*, *Stegocephalus inflatus* Krøyer and *Parandaniexis* sp. (cf. *mirabilis*) to X-ray microanalysis and those of *Stegocephaloides christianiensis* to electron diffraction analysis. We have measured the iron content of *S. christianiensis* by atomic absorption spectrophotometry and compared these data with results we have derived from a range of other amphipods from widely different habitats. These results are detailed below and an hypothesis is put forward which attempts to explain the function of these crystals in terms of the feeding habits of this enigmatic family.

Considerable interest has been generated in recent years on the subject of metal-containing granules in invertebrate tissues (see Coombs & George (1978), table 1 and reviews by Mason & Nott (1981); Brown 1982). Despite iron being the most abundant 'heavy' metal in the sea, least is known about iron-containing granules (Brown 1982) and comparatively few data exist on the concentration and use of this metal by crustaceans (White 1982).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

2. Material and methods

Live *Stegocephaloides christianiensis* were collected using a small mesh (*ca.* 1 mm) epibenthic dredge (D-net), towed at about 60–70 m over muddy sand bottoms either off Fintray Bay, Isle of Cumbrae or Kilchattan Bay, Isle of Bute (Firth of Clyde).

Museum material stored in 70% (by volume) alcohol was dissected, a midgut caecum removed, teased apart and stained by Perl's method as a squash preparation using acidified potassium ferrocyanide for ferric iron (Prussian Blue). Permanent preparations were cleared and mounted in polyvinyl lactophenol.

Material for investigation of fine structure was fixed in 4% (by volume) glutaraldehyde (fresh) in sodium cacodylate buffer (pH 7.2) made isotonic to seawater with sucrose (1 h 30 min with two washes at room temperature). Specimens were then washed in isotonic cacodylate buffer overnight (at room temperature), osmicated with osmium tetroxide in cacodylate buffer (10 g l^{-1}, 1 h, room temperature), and dehydrated through 10%, 30% to 70% (by volume) ethanol. Tissues were cleared in propylene oxide and embedded in TAAB resin before sectioning at 40 nm on a Huxley Mk II ultramicrotome, staining in uranyl acetate–lead citrate and examination in a Jeol 100C transmission electron microscope (t.e.m.). The presence of crystals made the cutting of perfect ultrathin sections difficult.

Fresh *S. christianiensis* material for X-ray microanalysis was similarly fixed in 4% (by volume) glutaraldehyde in cacodylate buffer and taken to 70% (by volume) ethanol. Some of this material was returned down a graded series of alcohols to cacodylate buffer before post-osmicating for 2 h in osmium tetroxide (10 g l^{-1}) in cacodylate and returning to 70% (by volume) ethanol. Tissues were then cleared and embedded as above, before sectioning at 0.5 μm onto aluminium support grids and examination in the Jeol 100C t.e.m. equipped with Link system EDX energy dispersive X-ray microanalyser. The remaining *S. christianiensis* material was similarly treated except that post-osmication was omitted.

Alcohol-fixed museum material of *Stegocephaloides*, *Stegocephalus* and *Parandaniexis* was transferred to 70% (by volume) ethanol without post-osmication and cleared, embedded and sectioned as above before X-ray microanalysis.

Alcohol-fixed material of *S. christianiensis* was similarly prepared without post-osmication and examined in a Jeol 100CX with s.t.e.m. attachment for X-ray distribution mapping (at Jeol U.K. Ltd, Colindale, London). Similar material was examined in a Jeol 100CX with electron diffraction.

S. christianiensis material for scanning electron microscopy (s.e.m.) (some partially dissected) was fixed in glutaraldehyde and osmium tetroxide (as above for fine structure), air dried and sputter coated with a gold–palladium mixture in a Polaron sputter coater and examined in a Jeol JSM 35 scanning electron microscope.

Iron determinations were made as follows. Freshly caught amphipods were kept in clean seawater (24–48 h, 4 °C) to void gut contents. They were then, individually, briefly double-washed in distilled water and transferred to preweighed aluminium foil planchettes, dried in an oven at 60 °C (72 h) and reweighed on a Beckman LM-500 microbalance. Cnidarians and pycnogonids were dried and weighed on a Mettler H51 balance. Individuals were transferred to separate acid-washed tubes to which Aristar nitric acid (B.D.H. Chemicals Ltd, Poole, Dorset) was added for acid digestion at 100 °C. Acid digests were made up to volume with distilled water and analysed for iron on a Varian AA-375 atomic absorption spectrophotometer with flame atomization and background correction.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

3. Crystal occurrence and composition

Table 1 lists the stegocephalid amphipods investigated. Crystalline inclusions were present in the midgut caeca cells of most species, though not in *Andaniopsis nordlandica*, *Tetradeion crassum*, *Andaniexis abyssi* or *Phippsiella similis*. Crystals in the other species were generally octahedral in shape and of a comparable size, with the exceptions of *Stegocephalus inflatus* (in which small rod-shaped crystals were as abundant as octahedra), and *Phippsiella* sp.G1 (see table 1 for explanation) in which most crystals were unusually large and rod-shaped (small crypts of octahedral crystals however occurred in the tissues of this species also). Most crystals stained strongly for Fe, although the reaction of *Stegocephaloides vanhoffeni* crystals was equivocal. Gut caeca tissue of *Andaniexis abyssi* gave a positive reaction for iron in the absence of discernible crystals.

Table 1. Stegocephalid amphipods investigated for crystalline inclusions in ventral gut caeca cells (proximal region) and the crystals' reaction for iron

species	crystal shape	crystal height × crystal breadth ± s.d.§ μm	iron reaction
Andaniopsis nordlandica (Boeck)	no crystals		−
Tetradeion crassum (Chilton)	no crystals		−
Andaniexis abyssi (Boeck)	no crystals		+ (tissue)
Bathystegocephalus inflatus (Walker)	octahedral–cubic?		+
Euandania ingens (Chevr.)	octahedral	22.77 ± 4.49 × 12.55 ± 4.10	+
Parandania boecki (Stebb.)	octahedral	6.60 ± 2.39 × 4.04 ± 0.83	+
Stegocephaloides christianiensis Boeck†	octahedral	23.15 ± 4.15 × 11.43 ± 2.65	+
S. auratus Sars	octahedral	16.49 ± 1.52 × 8.51 ± 1.12	+
S. vanhoffeni Schell.	octahedral	32.45 ± 5.00 × 15.96 ± 3.00	weak
Stegocephalus inflatus Krøyer	octahedral	23.45 ± 3.79 × 14.74 ± 2.76	+
	rod shaped	26.98 ± 3.97 × 8.79 ± 0.52	+
Phippsiella sp. O	octahedral	22.23 ± 5.61 × 10.96 ± 2.79	+
Phippsiella similis (Sars)‡	no crystals		−
Phippsiella sp. G1	rod shaped	67.00 ± 5.23 × 13.62 ± 1.49	+
	octahedral	19.04 ± 2.15 × 8.08 ± 1.43	+
Parandaniexis sp. cf. *mirabilis*	octahedral	36.94 ± 9.09 × 17.02 ± 8.52	+

† See Moore (1979); ‡ referred to as *Stegocephalus similis* by Moore (1979); § $n = 10$.
Taxonomic difficulties remain in certain of these cases, particularly relating to *Phippsiella* (see Steele 1967) and specific attribution remains questionable in some of the entities examined.

From the appearance of squash preparations (both fresh and fixed) and of thin sections (see below), Fe cells contain only a single crystal each in all species. The size of the crystals consistently increased proximally towards the junction of the caeca with the intestine in all species (see Moore 1979), though none was visible, at the light microscope level, in the most distal region of the caecum ('juvenile zone', *sensu* Martin 1964).

In view of the general dearth of museum material from this little-known, largely bathyal family, attention was concentrated on the small species *Stegocephaloides christianiensis*, which was locally available in the field, in an attempt to characterize the crystals' chemical composition further. Comparison was then made with crystals from the next most available species *Stegocephalus inflatus* and with the large species *Parandaniexis* sp. (cf. *mirabilis*) (both from museum collections).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(a) *Stegocephaloides christianiensis*

(i) *Caecum ultrastructure*

Each caecum is a blind-ending sac with walls one cell thick. Mixing and expulsion of the lumen contents are achieved by contraction of spiral muscle fibres external to the basement membrane (figure 1, plate 1). Proximal to the embryonic (E) cells at the blind end, two cell facies are distinguishable. One has a brush border of long microvilli impinging on the lumen, the other has a luminal border of short, stubby microvilli. The former is identified with the R/F-cells of Icely (1981), the latter with his B-cells (see also Schultz 1975). No evidence has been discovered which suggests that R- and F-cells of separate function exist (cf. decapod situation reviewed by Gibson & Barker 1979). Rather it appears that cells generated from the distal embryonic zone mature proximally and subsume a variety of functions (secretion, absorption, storage) though perhaps to different degrees in different regions of the caecum. Some of these cells ultimately mature into B-cells which are responsible for the bubbly inside appearance of the proximal region of the caecum. These cells appear to degenerate, releasing their contents either by apocrine (Schultz 1975) or holocrine (Shyamasundari & Varghese 1973) secretion. Summary descriptions follow.

R/F-cells (= *Fe cells*). (See figures 1–4, plate 1, figure 5, plate 2). Columnar cells, up to *ca.* 70 μm high, with terminal brush border of long (*ca.* 2 μm) microvilli; with centrally located, sub-spherical nuclei (*ca.* 8 μm in diameter); cytoplasm containing abundant mitochondria with numerous cristae, extensive rough endoplasmic reticulum, curved Golgi bodies with few cisternae; with basal osmophilic lipid globules; Fe crystals not membrane bound; cell base bounded by basal membrane. Small sub-spherical calcium granules (see below) also present.

B-cells. (See figures 2, 3 and 6). Tall cells (*ca.* 80 μm), apically distended with a luminal border of short, electron-dense microvilli (*ca.* 0.3 μm). Apical complex of membrane-bound vesicles of varying degrees of coalescence, containing electron-dense or electron-lucent material. Intravacuolar spaces with electron-dense rosettes of glycogen. Intervacuolar cytoplasm packed with rough endoplasmic reticulum. Basal nucleus ovoid, cells narrow basally.

Calcium granules. (See figures 4, 7*a* and 9–14, plate 3). Small (*ca.* 0.5 μm) sub-spherical granules. X-ray microprobe analysis (figures 7*a* and 8) and X-ray distribution scan (figures 9–14) showed Ca and S to be consistently present with variable quantities of Si (sometimes dominant), P (always less than S) and Fe. Dense background cytoplasm (figure 7*b*) showed small P, Si, Ca, S and Fe peaks while pale background cytoplasm (figure 7*c*) showed Si, S, Cl and P. Minor peaks of Cu, Zn and Pb (figures 7 and 8) may reflect contamination (see below) or background.

(ii) Fe-*crystal structure and composition*

The elemental spectra derived from X-ray microprobe analysis of a number of Fe crystals in recently alcohol-fixed material revealed strong Fe peaks (see for example, figure 15*a*), with minor peaks for Si, P, S, Cl, K, Ca, Cu and Zn. These minor peaks were also present in background cytoplasm adjacent to the crystals. The Al peak which recurred universally is a product of the support grid. Figure 16 (plate 4) shows the X-ray distribution of Fe across the field of figure 17. Clearly, iron is localized within the cell in the Fe crystals and is not grid background (cf. Icely & Nott 1980). Scans for Si (figure 18), Ca (figure 19) and P showed no localization of these elements in the Fe crystal. These elements, together with S, Cl, K, Cu and Zn were identified in the surrounding cytoplasm by X-ray microanalysis. Crystal composition is homogeneous with no distinctive nucleus or core.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Since comparisons follow using museum material stored for varying (sometimes considerable) lengths of time, the crystal composition of *S. christianiensis* museum-stored since 1893 was compared with the above. Figure 20 shows the same Fe, Cu and Zn peaks, but the lighter elements (P, S, Cl, Ca) were missing and consistent Pb peaks emerged where their presence was very spasmodic and weak before (beyond the region of the spectrum illustrated in figure 7*a*). Possible Ca granules also showed a Pb peak but lipid material in the same cells gave Al, Cu and Zn with no Pb peak. The background cytoplasm was too diffuse to count effectively. It is thought that Pb may have leached with time from glass storage vessels and that its presence has probably been exaggerated owing to replacement. Iron, however, is clearly unaffected by long-term storage in ethanol.

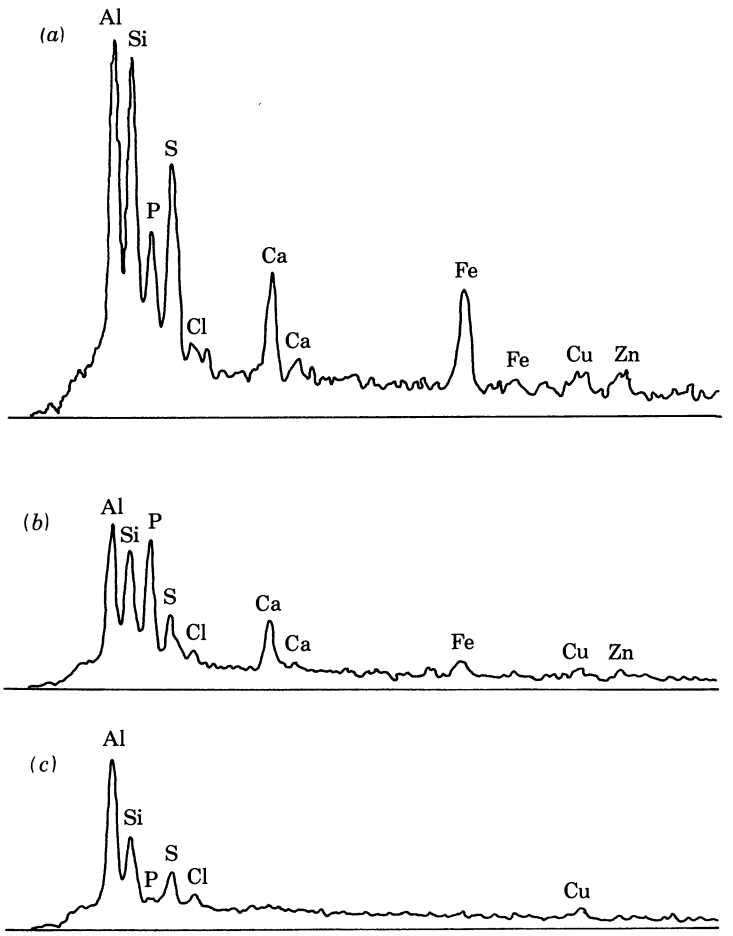

FIGURE 7. *Stegocephaloides christianiensis*, ventral caecum R/F cell (tissue fixed in 70% alcohol). Spectra of elemental analyses of (*a*) calcium granule, (*b*) dense cytoplasm adjacent to granule and (*c*) pale cytoplasm adjacent to granule, vertical scale is an arbitrary number of counts, horizontal scale is a relative scale of X-ray energies. Peaks for Al originate from support grid, minor peaks of Cu, Zn and Pb may reflect contamination or background. This calcium granule has high silicon (cf. figure 8, plate 3).

Figure 21 confirms that the Fe crystals are not membrane bound: they abut directly against granular cytoplasm which is presumably involved in crystal formation. At magnifications above *ca.* 50000×, crystal ultrastructure emerges. The crystal is seen (figure 22) to consist of hexagonally arranged, electron dense cores of 5.8 ± 0.3 nm diameter at intercore distances of 10.5 ± 0.5 nm, 7.5 ± 0.5 nm and 9.5 ± 0.5 nm. The crystal lattice has plane spacings estimated

from small angle electron diffraction (360 cm camera length) of 9.2 ± 0.3 nm, 7.2 ± 0.3 nm and 7.0 ± 0.3 nm (figure 23). High power transmission electron microscopy (150000×) resolved the following plane spacings, 8.75 ± 0.3 nm, 7.4 ± 0.3 nm and 6.7 ± 0.3 nm. These two estimates agree closely. So the plane spacings of the lattice can be given as 9.0 ± 0.5 nm, 7.3 ± 0.5 nm and 6.9 ± 0.5 nm. Wide angle electron diffraction patterns (camera length, 49 cm) of the cores gave four rings with *d* spacings of 0.250, 0.223, 0.191 and 0.145 nm (all ± 0.003 nm) (figure 24). These data show conclusively that the crystals consist of ferritin (Harrison *et al.* 1967; Harrison 1981), crystals of which consist of regularly arranged cores of iron hydroxide and phosphate with a random arrangement within each electron dense core. Professor P. Harrison (Biochemistry Department, University of Sheffield) has been kind enough to examine our electron diffraction photographs and agrees with our identification of ferritin.

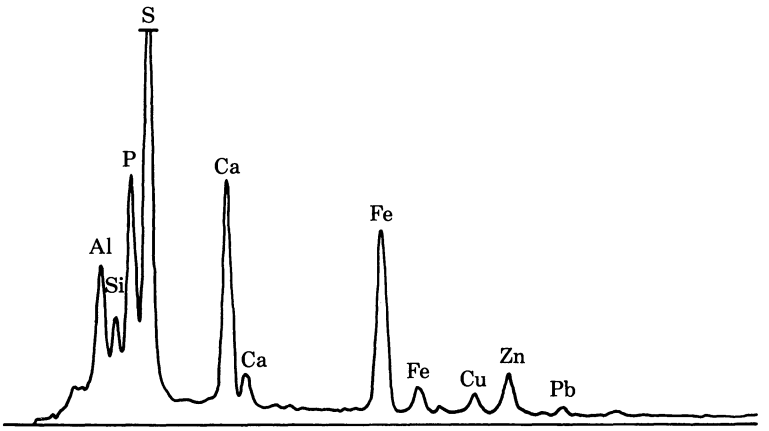

FIGURE 8. *Stegocephaloides christianiensis*, ventral caecum R/F cell (tissue fixed in 70% alcohol). Spectrum of elemental analysis of calcium granule showing relatively minor silicon peak and an additional minor (background?) peak for lead (cf. figure 7*a*). Identity of axes as for figure 7.

(*b*) *Stegocephalus inflatus*

X-ray microanalysis of Fe crystals revealed peaks (figure 15*b*) in the following elements, Al, Si, P, S, Ca and Fe with sometimes also traces of Cu and Zn. Al (see above) is background to the instrument. Si, S, Ca and Cl were identified in the surrounding cytoplasm, with perhaps a little phosphorus.

The smaller, amorphous calcium granules consistently showed strong peaks for Ca and P (figure 25*a*). In contrast to *Stegocephaloides christianiensis*, however, the peaks for P were always stronger than for S. Si and Cl were present in background cytoplasm. Some granules showed traces of Cu and Zn and one had a small Fe peak.

(*c*) *Parandaniexis* sp. (cf. *mirabilis*)

X-ray microanalysis of Fe crystals revealed peaks (figure 15*c*) in Al, Si, P, S, Fe with traces of Cu and Zn. The Fe peaks dominated the spectrum. Only Al (grid) and Si were identified in the cytoplasm. Small, amorphous calcium granules showed strong peaks for Ca and P with smaller peaks for Si, S and Cl (figure 25*b*).

Based on crystal size, shape, colour, elemental and ultrastructural composition we propose that the Fe crystals in *S. inflatus* and *Parandaniexis* sp. are made up of the same material as those in *Stegocephaloides christianiensis*, that is, ferritin.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

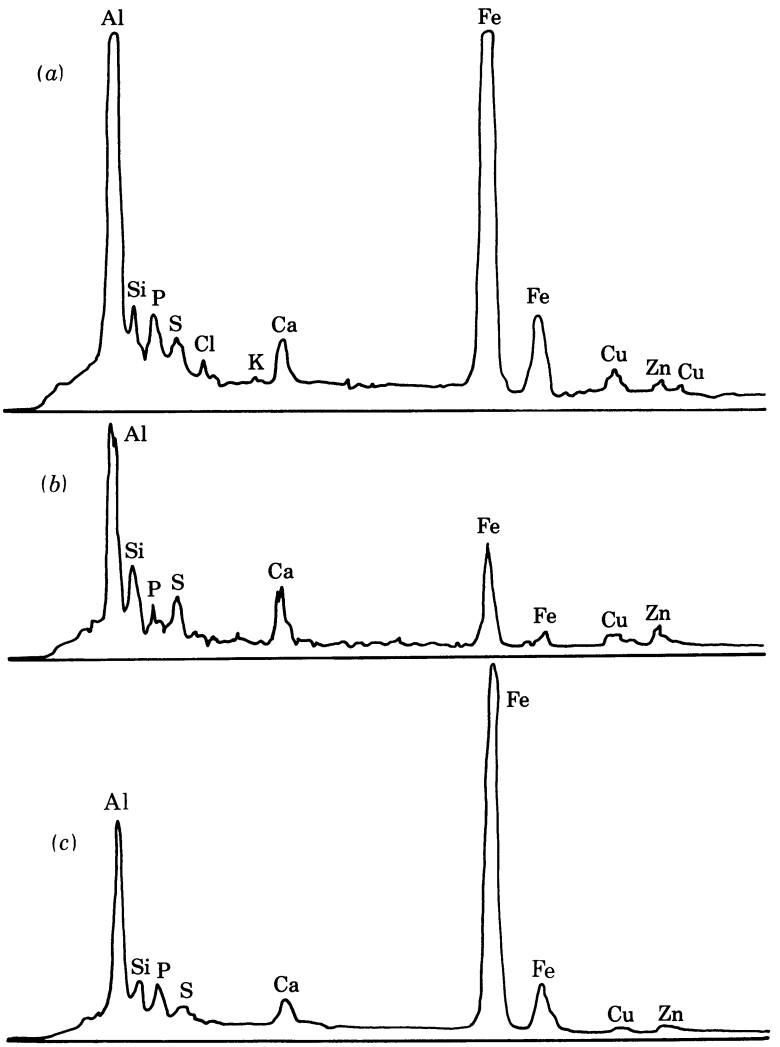

FIGURE 15. Spectra of elemental analyses of Fe crystals in R/F cells of alcohol-fixed ventral caeca of (*a*) *Stegocephaloides christianiensis* (recently fixed), (*b*) *Stegocephalus inflatus* and (*c*) *Parandaniexis* sp. (cf. *mirabilis*), showing strong Fe peaks in all cases and variably present minor peaks for Si, P, S, K, Ca, Cu and Zn which are also present in the background cytoplasm of the R/F cell (see text for details). Strong Al peak is a product of the support grid. Identity of axes as for figure 7.

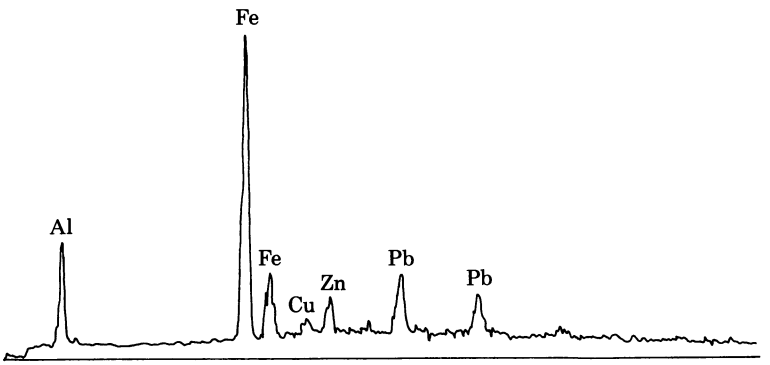

FIGURE 20. *Stegocephaloides christianiensis* ventral caecum R/F cell Fe crystal. Spectrum of elemental analysis in museum material alcohol-fixed in 1893. In comparison with figure 7*a* peaks for P, S, Cl, Ca are missing. Pb peaks are now apparent in the spectrum beyond the highest energies included in figure 7*a*. Identity of axes as for figure 7. Scale extended horizontally.

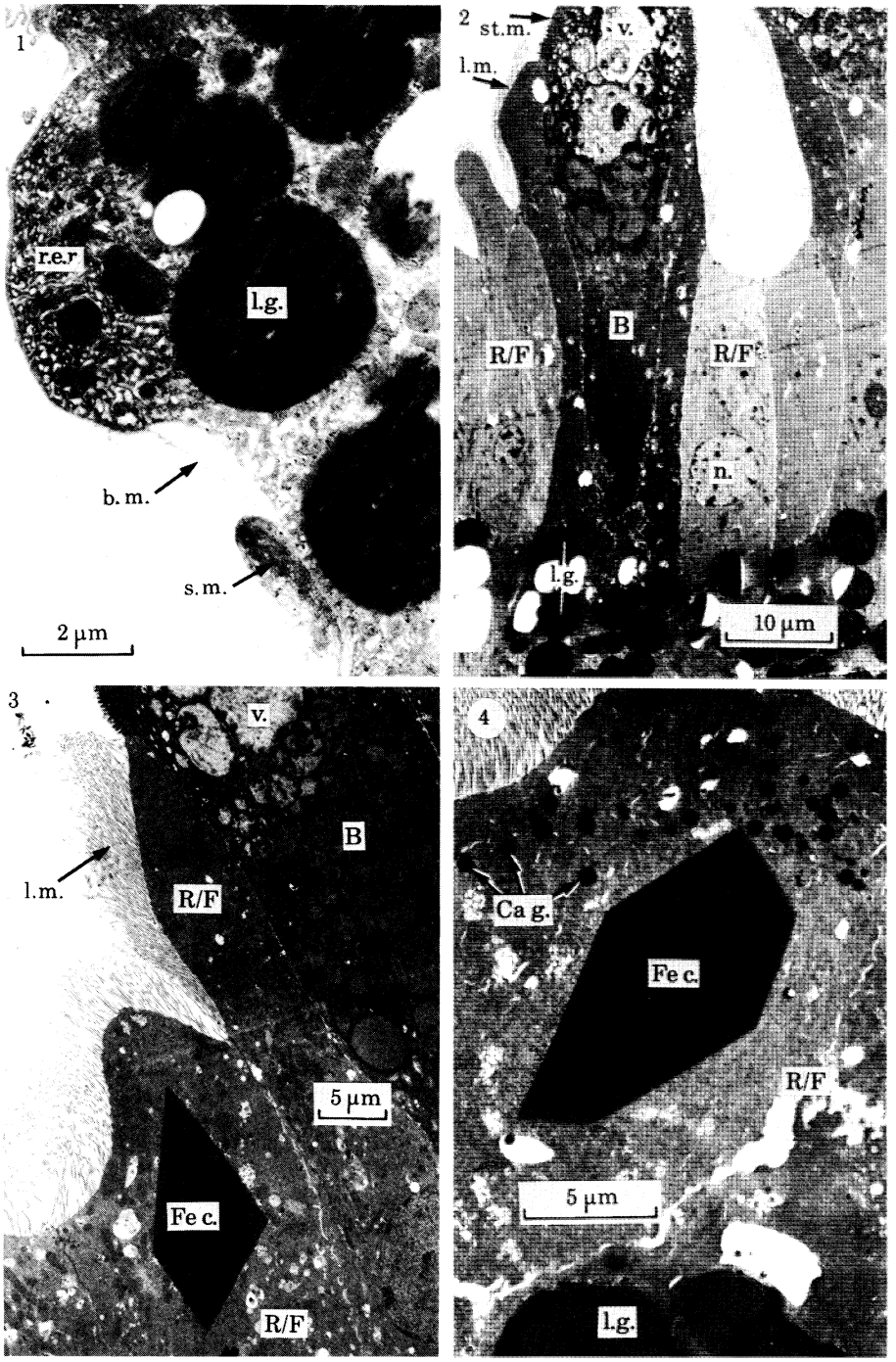

FIGURE 1. *Stegocephaloides christianiensis*, ventral caecum. Basal region of R/F cell with osmophilic lipid globules (l.g.) and dense rough endoplasmic reticulum (r.e.r.), showing discrete spiral muscle (s.m.) below the basement membrane (b.m.). Magn. ×7000.

FIGURE 2. *Stegocephaloides christianiensis*, ventral caecum. Epithelium consists of two cell types: R/F cells with long microvilli (l.m.), sub-spherical nuclei (n.) and osmophilic lipid globules (l.g.) basally, some of which have been lost in sectioning; tall B cells (forming epithelial crests) with stubby microvilli (st.m.), apical complex of membrane-bound vesicles (v.) and basal ovoid nucleus. Magn. ×1400.

FIGURE 3. *Stegocephaloides christianiensis*, ventral caecum. Apical regions of R/F cell showing Fe crystal (Fe c.) and long microvilli (l.m.), and B cell showing apical complex of membrane bound vesicles (v.). Magn. ×1800.

FIGURE 4. *Stegocephaloides christianiensis*, ventral caecum. R/F cell showing Fe crystal (Fe c.), apical calcium granules (Ca g.) and basal lipid globules (l.g.). Magn. ×3600.

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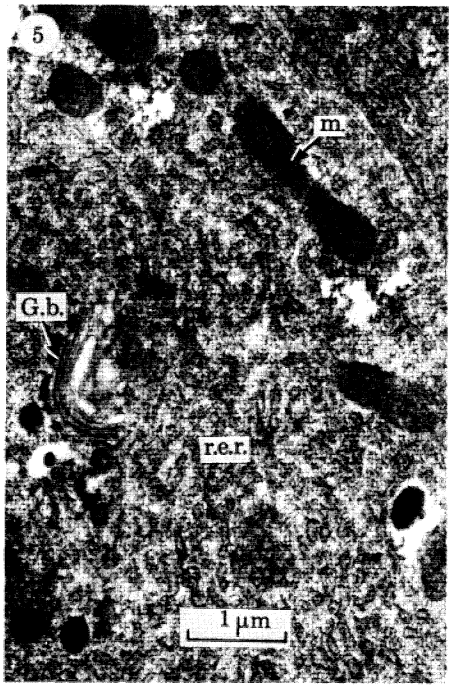
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



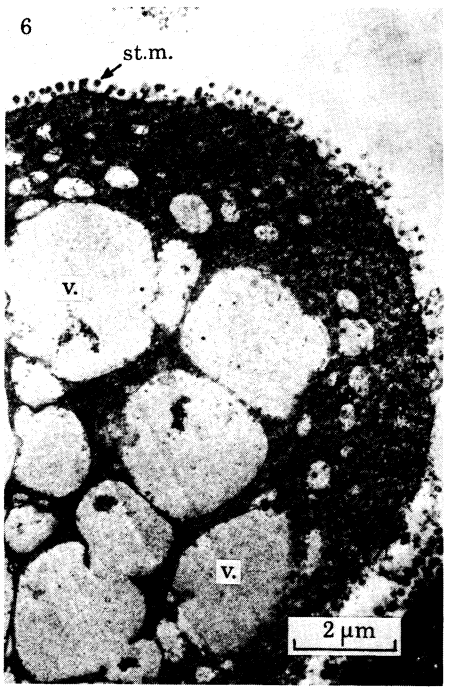


FIGURE 5. *Stegocephaloides christianiensis*, ventral caecum. R/F cell showing curved Golgi body (G.b.), dense rough endoplasmic reticulum (r.e.r.) and abundant mitochondria (m.). Magn. × 13000.

FIGURE 6. *Stegocephaloides christianiensis*, ventral caecum. Apical region B cell showing stubby microvilli (st.m.) and apical complex of coalescing membrane-bound vesicles (v.) with mostly electron-lucent contents. Magn. × 7000.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

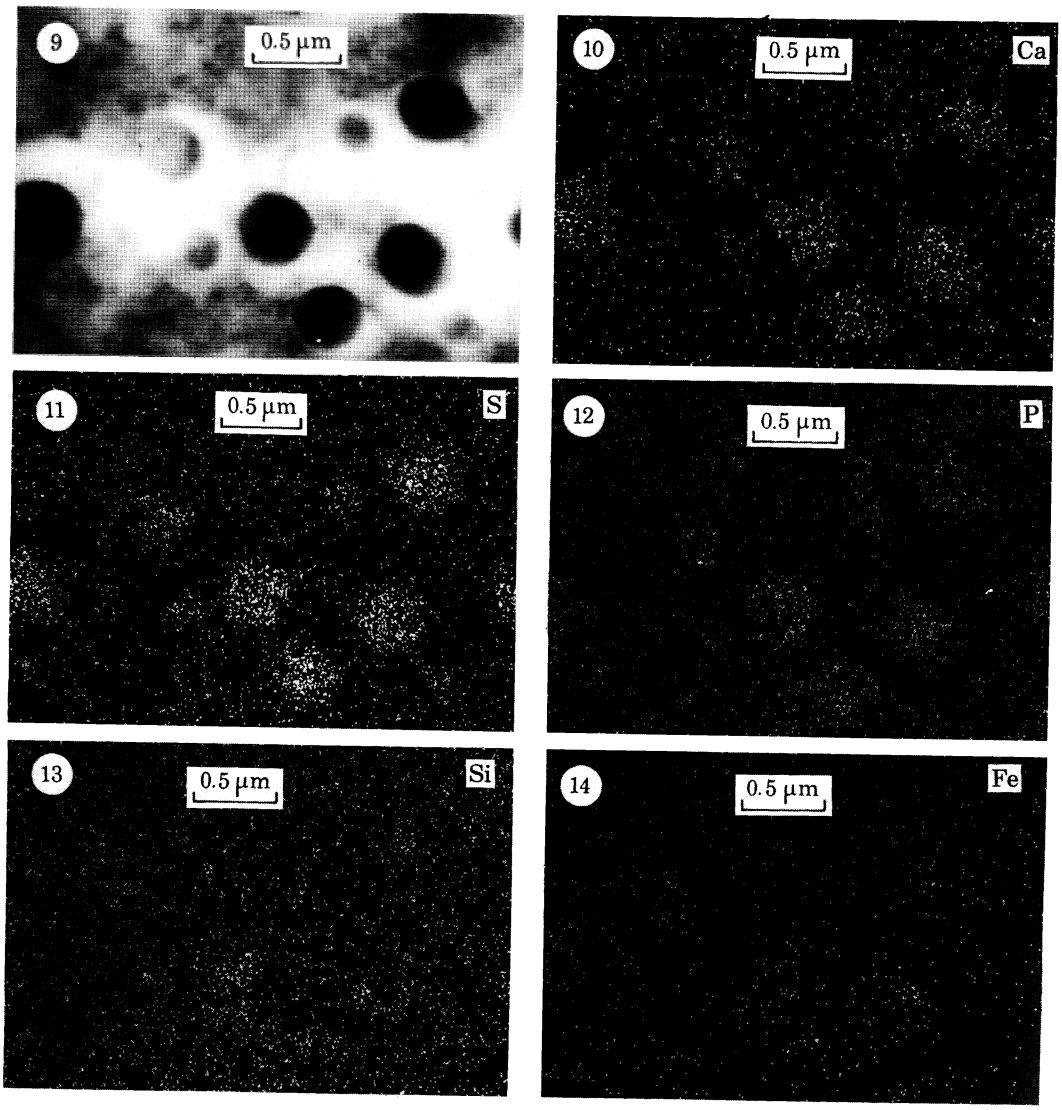
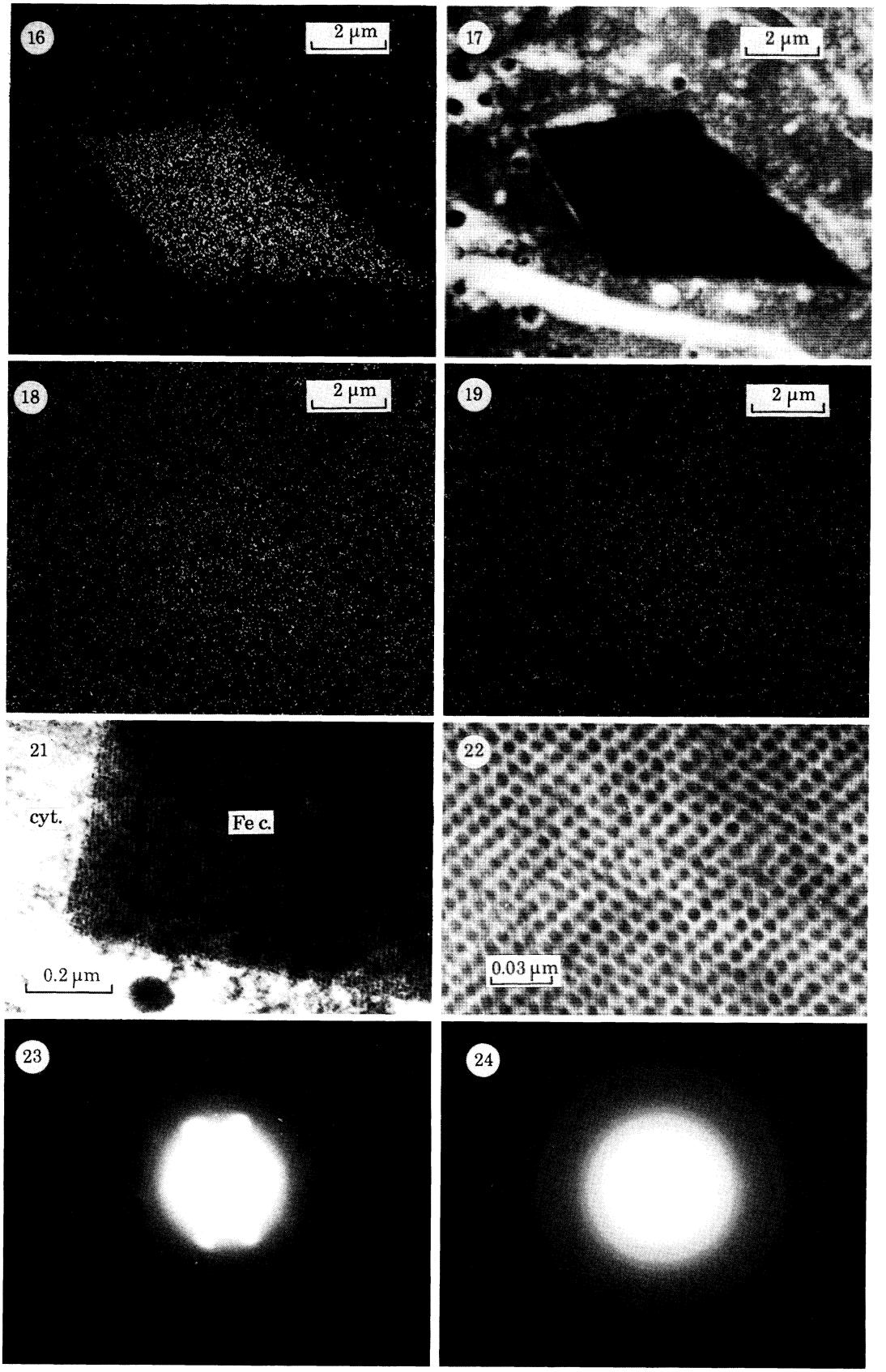


FIGURE 9. *Stegocephaloides christianiensis*, ventral caecum, s.t.e.m. micrograph of alcohol-fixed R/F cell calcium granules. Magn. ×22000.

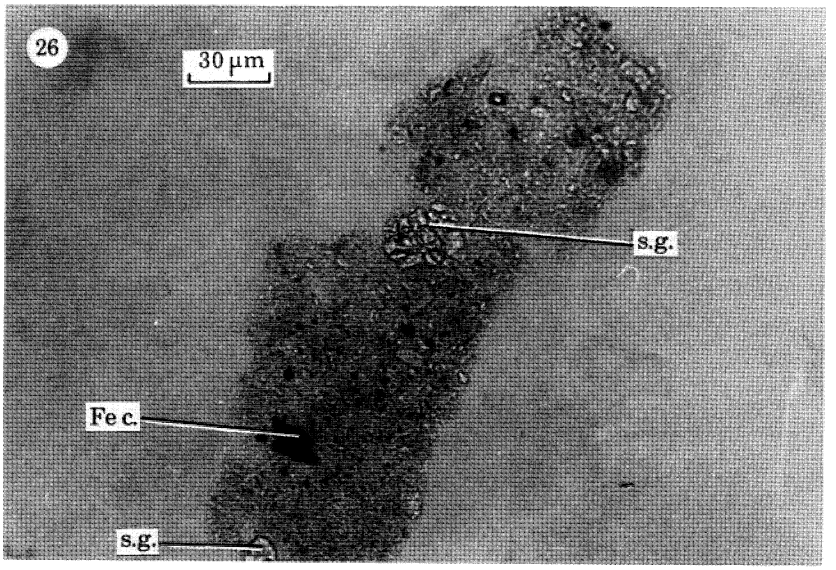
FIGURES 10–14. *Stegocephaloides christianiensis*, ventral caecum R/F cell calcium granules. X-ray distribution images for calcium (Ca) (figure 10), sulphur (S) (figure 11), phosphorus (P) (figure 12), silicon (Si) (figure 13) and iron (Fe) (figure 14) over the same area as figure 8 showing calcium, sulphur and (to a lesser extent) phosphorus to be localized within the granules but silicon and iron to be background. Magn. ×22000.

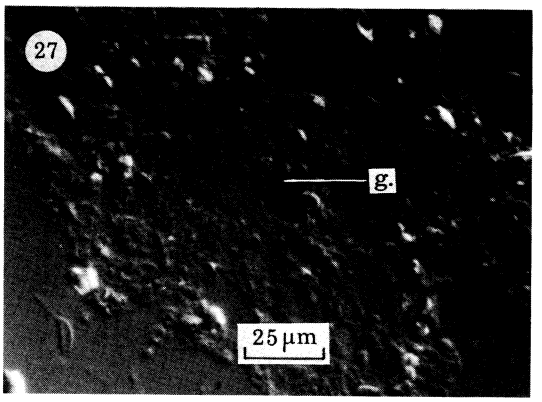
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

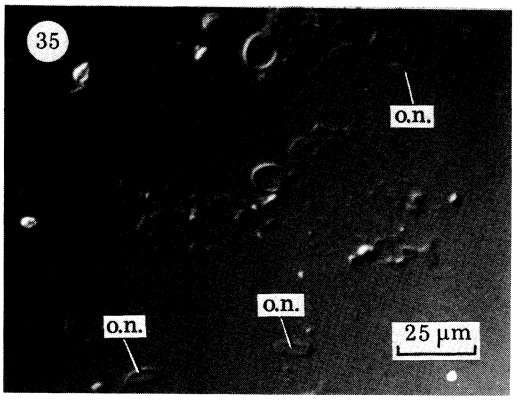


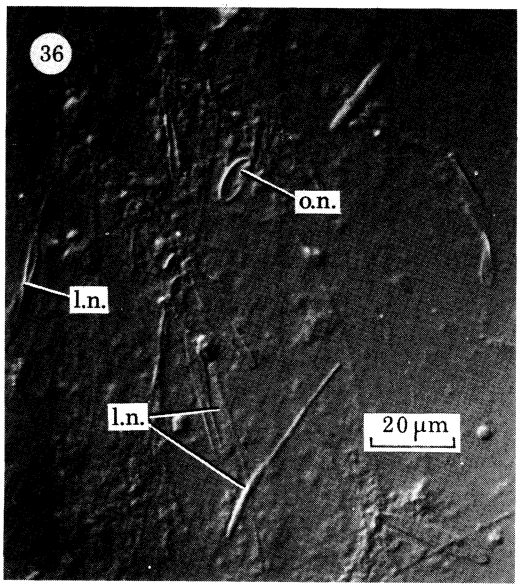
FIGURES 16–19, 21–24. For description see p. 227.

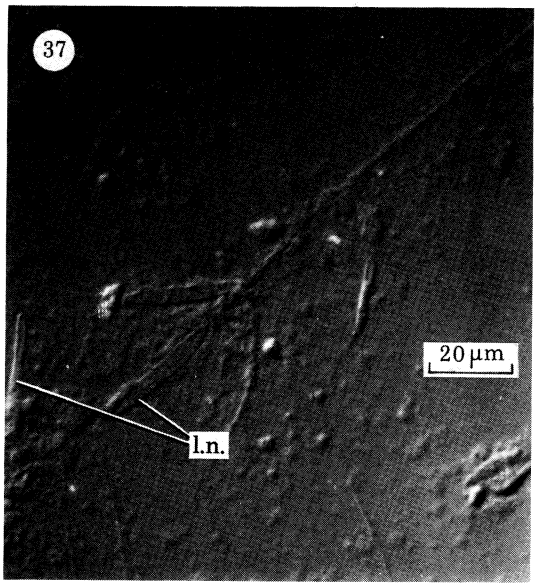
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





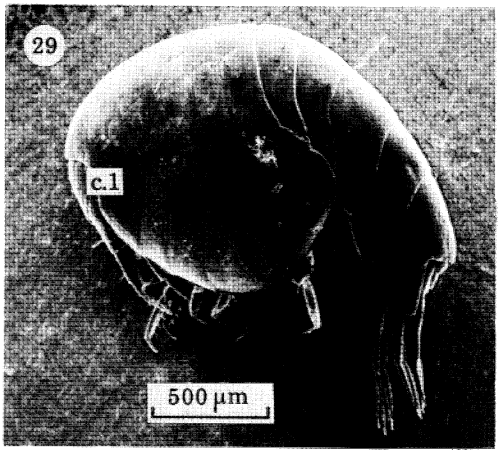


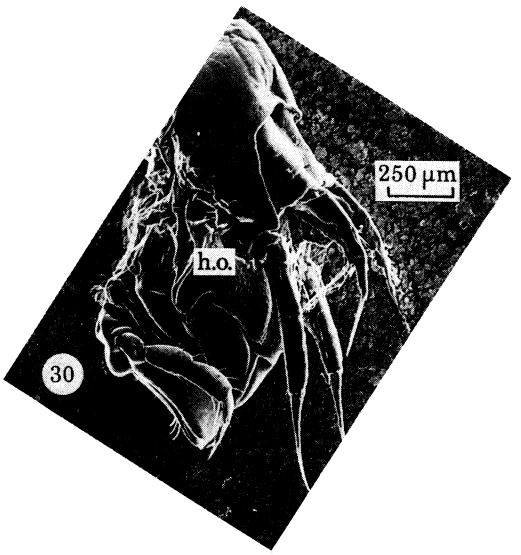


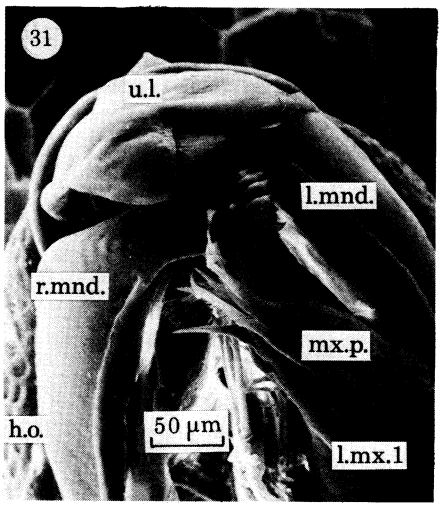


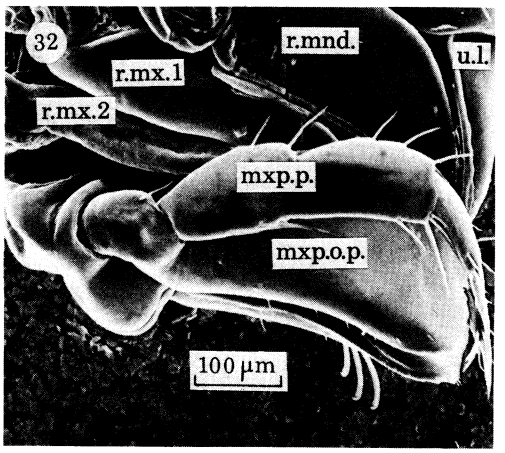
FIGURES 26, 27, 35–37. For description see p. 227.

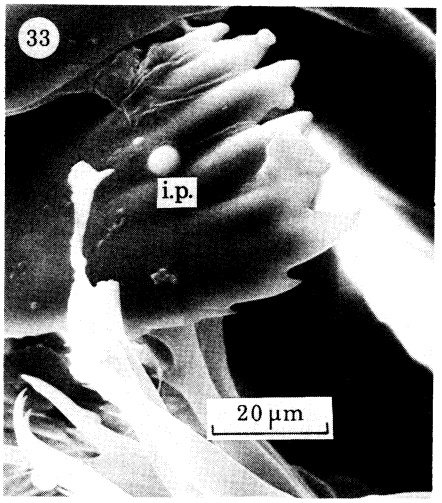
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

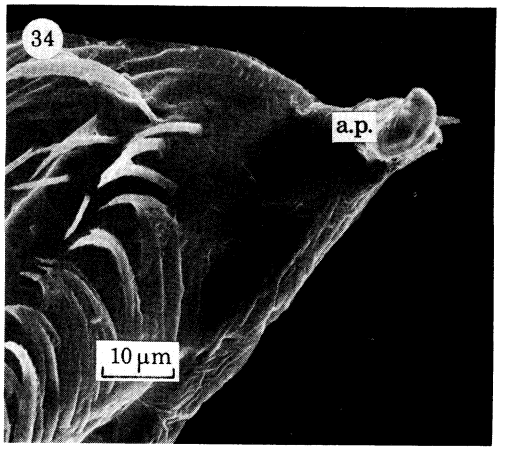












FIGURES 29–34. For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The calcium granules in the three species investigated showed variation in the proportions of constituent elements present in addition to calcium. Higher peaks for P rather than for S were features of the elemental spectra in museum material of *Stegocephalus* and *Parandaniexis*. Fresh alcohol-fixed material of *Stegocephaloides christianiensis*, however, yielded higher peaks for S than P. Since the examination of the very old *S. christianiensis* material showed that elemental composition of preserved tissue may change with long-term storage in fixative, these variations probably do not imply any real difference in the composition of the Ca granules of the different species.

Description of plate 4

Figures 16–19. *Stegocephaloides christianiensis*, ventral caecum R/F cell, Fe crystal. X-ray distribution images for iron (figure 16), silicon (figure 18) and calcium (figure 19) over the area shown in the s.t.e.m. micrograph (figure 17) of an iron crystal with surrounding cytoplasm. Magn. ×6000.

Figure 21. *Stegocephaloides christianiensis* ventral caecum R/F cell, Fe crystal (Fe c.) with no bounding membrane, surrounded by granular cytoplasm (cyt.). Magn. ×70000.

Figure 22. *Stegocephaloides christianiensis* ventral caecum R/F cell, Fe crystal showing hexagonally arranged electron dense cores. Magn. ×340000.

Figure 23. *Stegocephaloides christianiensis* ventral caecum R/F cell, Fe crystal. Small angle electron diffraction pattern (camera length, 360 cm) showing six strong spots with plane spacings of 9.2 nm, 7.2 nm and 7.0 nm (all ±0.3 nm).

Figure 24. *Stegocephaloides christianiensis* ventral caecum R/F cell, Fe crystal. Wide angle electron diffraction pattern (camera length, 49 cm) of cores with four rings with d spacings of 0.250, 0.223, 0.191 and 0.145 nm (all ±0.003 nm).

Description of plate 5

Figure 26. *Stegocephaloides christianiensis* light micrograph of faecal pellet showing sand grains (s.g.) and Fe crystal (Fe c.) (squash preparation stained by Perl's method). Magn. ×350.

Figure 27. *Stegocephaloides christianiensis*. Nomarski optics micrograph of a squash preparation (unstained) of fresh stomach contents, showing an irregularly shaped red granule (g.) in the midst of amorphous material. Magn. ×400.

Figure 35. *Stegocephaloides christianiensis*. Nomarski optics micrograph of squash preparation (unstained) of fresh stomach contents, showing small, oval nematocyts (o.n.). Magn. ×400.

Figure 36. *Stegocephaloides christianiensis*. Nomarski optics micrograph of squash preparation (unstained) of fresh stomach contents, showing long (l.n.) and oval nematocyts (o.n.). Magn. ×550.

Figure 37. *Stegocephaloides christianiensis*. Nomarski optics micrograph of squash preparation (unstained) of fresh stomach contents, showing long nematocyts (l.n.). Magn. ×550.

Description of plate 6

Figure 29. *Stegocephaloides christianiensis*. Scanning electron micrograph (s.e.m.) of whole animal viewed from left side. Note: head is obscured by first coxal plate (c.1). Magn. ×30.

Figure 30. *Stegocephaloides christianiensis*. S.e.m. of head viewed from right side. Note: honeycomb ornamentation (h.o.) of base of mandible. Magn. ×32.

Figure 31. *Stegocephaloides christianiensis*. S.e.m. of mouthpart bundle (ventral view) with maxillipeds and right first and second maxillae removed to reveal right mandible (r.mnd.), left first maxilla (l.mx.1) with maxillary palp (mx.p.). Left mandible (l.mnd.) obscured beneath asymmetrical, bilobed upper lip (u.l.). Magn. ×200.

Figure 32. *Stegocephaloides christianiensis*. S.e.m. of mouthpart bundle from right side showing maxillipedal outer plate (mxp.o.p.), maxillipedal palp (mxp.p.), upper lip (u.l.), right mandible (r.mnd.), right first maxilla (r.mx.1) and right second maxilla (r.mx.2). Magn. ×110.

Figure 33. *Stegocephaloides christianiensis*. S.e.m. of incisor process (i.p.) of right mandible. Magn. ×750.

Figure 34. *Stegocephaloides christianiensis*. S.e.m. of apex of outer lobe of inner lip, showing apical papilla (a.p.) and field of inwardly recurved setae on inner face. Magn. ×900.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

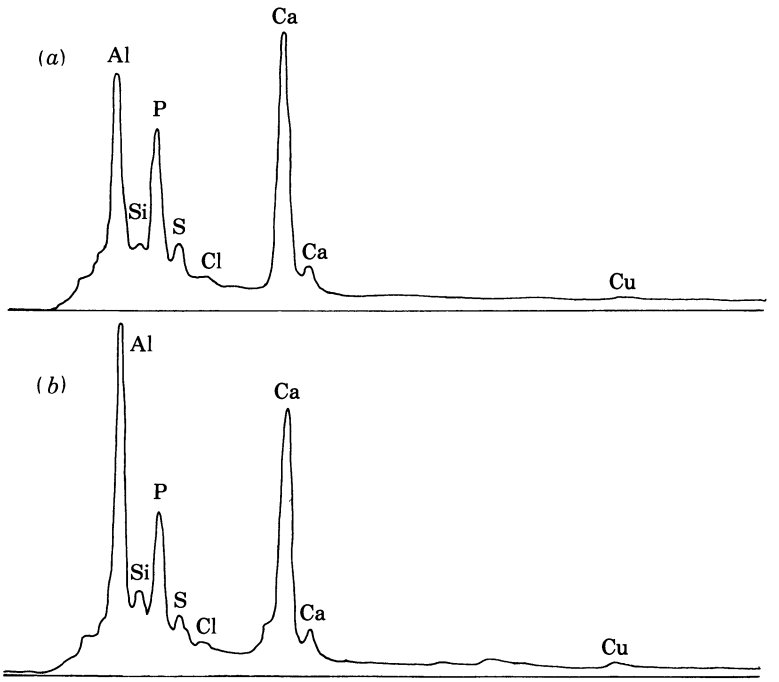


FIGURE 25. Spectra of elemental analyses of calcium granules in alcohol-fixed R/F cells of ventral caeca of (*a*) *Stegocephalus inflatus* and (*b*) *Parandaniexis* sp. (cf. *mirabilis*) showing strong peaks for Ca and P and minor peaks for Si, S and Cl. Al peak is product of support grid. Identity of axes as for figure 7.

4. THE FUNCTION OF FERRITIN CRYSTALS IN *STEGOCEPHALOIDES CHRISTIANIENSIS*

The high concentration of Fe in the crystals of the three species above (as deduced from peak height on the energy dispersion spectra) and the widespread occurrence of such crystals only within the confines of the Stegocephalidae among the Amphipoda (table 1) clearly suggest a particular adaptive significance.

To investigate whether crystals were voided with the faeces (see Moore 1979) live *Stegocephaloides christianiensis* were transferred to a small jar containing filtered seawater (0.4 μm Whatman in-line filters) and kept at 4 °C in a refrigerator for 10 d. Faeces were collected and the animals' guts dissected out. Tissue samples and faeces were stained by Perl's method and examined microscopically. Figure 26 (plate 5) shows a faecal pellet containing sand grains and a blue-staining Fe crystal (crushed) within an unstained matrix of amorphous particles. The stomach lumen of starved animals was full of crystals and soft, red 'mush'. The crystals appeared redder in colour than the golden-brown ones embedded in the caeca. Many of the objects in the stomach were irregularly shaped granules rather than regular octahedra (figure 27). Those octahedral crystals in the stomach lumen, however, were of a size identical with crystals embedded in the proximal region of the gut caeca in both starved and fresh animals; so were the octahedral crystals in the faeces (figure 26) voided by non-feeding animals (table 2). These observations support the thesis that crystals are assembled intracellularly (one per Fe cell) in the ventral caeca, that they are gradually elaborated within these cells and propelled forwards by a cell cycle existing within each caecum (Martin 1969) from the blind end proximally, eventually either to be extruded or, more likely, sloughed-off with effete and dying

cells (Icely & Nott 1980), eventually gaining the stomach lumen, whence they are passed out of the body in the faeces. This hitherto unavailable (Moore 1979) evidence clearly points towards the crystals' role as being one of iron excretion; perhaps this is one of the processes by which the Fe content of the tissues is regulated.

TABLE 2. DIMENSIONS OF Fe CRYSTALS IN GUT REGIONS OF *STEGOCEPHALOIDES CHRISTIANIENSIS* UNDER DIFFERENT FEEDING REGIMES

material		crystal height × crystal breadth ± s.d. (μm)
freshly collected†		
distal region of caecum	($n = 10$)	8.13 ± 2.23 × 4.58 ± 0.83
proximal region of caecum	($n = 10$)	23.15 ± 4.15 × 11.43 ± 2.65
starved for 10 d		
distal region of caecum	($n = 10$)	12.98 ± 2.49 × 6.70 ± 1.00
proximal region of caecum	($n = 10$)	21.17 ± 4.61 × 12.87 ± 4.09
in stomach lumen	($n = 10$)	24.25 ± 4.72 × 13.09 ± 5.80
in voided faeces	($n = 3$)	23.05 ± 3.73 × 15.96 ± 6.47

† Data from Moore (1979).

5. THE CONCENTRATION OF Fe IN WHOLE AMPHIPODS

The concentration of iron in *Stegocephaloides christianiensis* was compared with that in a variety of other amphipod species representing the Suborders Hyperiidea (*Hyperia galba* (Montagu) taken from *Rhizostoma octopus* (L.) and *Cyanea capillata* (L.)), Caprellidea (*Caprella acanthifera* Leach) and Gammaridea from the following habitats: freshwater (*Gammarus pulex* (L.)); brackish water (*G. duebeni* Liljeborg); sandy shore (*Bathyporeia pilosa* Lindström); and rocky shore algae (*Hyale nilssoni* Rathke, *Microdeutopus versiculatus* (Bate), *Amphithoe rubricata* (Montagu), *Jassa falcata* (Montagu)); and from the following feeding types: detritivorous (*Orchestia gammarellus* (Pallas)); suspension feeding (*Ampelisca macrocephala* Liljeborg, *Melphidipella macra* (Norman)); together with other deep water benthic species of little known biology (*Epimeria cornigera* (Fabricius), *Eusirus longipes* Boeck and *Maera loveni* (Bruzelius)), all freshly collected from the Firth of Clyde.

Two collections of *Stegocephaloides christianiensis* were made in January 1982 (22 animals) and March 1982 (five). There was no significant difference between regression coefficients for the relations of lg body content against lg body mass in the two collections (table 3) and these data have been combined. Unless otherwise stated *S. christianiensis* results refer to combined data.

Iron content was non-linearly related to body dry mass in *S. christianiensis* (figure 28) according to the equation,

$$\lg [\mathrm{Fe}] = 1.7122 + 0.4530 \lg W (p < 0.001, \text{d.f. } 25) \quad (1)$$

where [Fe] = Fe content in micrograms and W = dry mass in grams, that is,

$$[\mathrm{Fe}] = 51.5466 W^{0.4530} \quad (2)$$

The relation between iron content and dry mass in all other marine amphipods combined (excepting data for the numerically dominant '*Hyperia galba* from *Cyanea*' which might

otherwise swamp any general amphipod trend: see table 3) (figure 28) is represented by the equation,

$$\lg [\mathrm{Fe}] = 2.0652 + 0.6503 \lg W (p < 0.001, \text{d.f. } 69) \quad (3)$$

If all iron was surface-adsorbed, say as a precipitate of hydroxide (Moore 1977), then

$$[\mathrm{Fe}] \propto \text{surface area}$$

that is,

$$\propto L^2,$$

that is,

$$\propto W^{\frac{2}{3}},$$

where L = length and W = mass. In such a case, the power function in (2) would be 0.6667. Alternatively, if surface adsorbed iron is of minor importance and

$$[\mathrm{Fe}] \propto W$$

then the power function in (2) would be 1.0.

TABLE 3. REGRESSION COEFFICIENTS (±95% CONFIDENCE LIMITS) FROM EQUATIONS DERIVED FROM LEAST SQUARES REGRESSION ANALYSIS

category	number of replicates	regression coefficient		95% confidence limits	difference from 0.6667	difference from 1.00
(i) *Epimeria cornigera*	6	0.6134	±	0.1830	n.s.	†
(ii) *Stegocephaloides christianiensis*						
(Jan)	22	0.4798	±	0.1927	n.s.	†
(Mar)	5	1.2916	±	0.5626	†	n.s.
(all)	27	0.4530	±	0.1567	†	†
(iii) all marine amphipods except *S. christianiensis* and *Hyperia* (from *Cyanea*)	71	0.6503	±	0.1302	n.s.	†
(iv) all marine amphipods including *S. christianiensis* but excepting *Hyperia* (from *Cyanea*)	98	0.5883	±	0.0565	†	†
(v) *Hyperia* from *Cyanea*	64	0.3387	±	0.1317	†	†

Lg iron content (in micrograms) of various categories of amphipods regressed on lg dry mass (in grams). n.s., Not significantly different ($p > 0.05$); †, significantly different ($p < 0.05$).

Table 3 lists the regression coefficients and 95% confidence limits from equations for Fe content regressed on body dry mass in those amphipods for which there was a significant ($p < 0.05$) regression. In the following categories, the regression coefficient was not significantly different ($p > 0.05$) from 0.6667: *Epimeria cornigera*, *Stegocephaloides christianiensis* (January) and 'all marine amphipods, except *S. christianiensis* and *Hyperia* (from *Cyanea*)'. Surface adsorption would explain the iron content of these categories (with smaller animals having a relatively higher iron concentration than larger individuals) and fits the data better than a direct mass effect. By contrast, *S. christianiensis* (March), *S. christianiensis* (all) and 'all marine amphipods including *S. christianiensis* but excepting *Hyperia* from *Cyanea*' and '*Hyperia* from *Cyanea*' each had functions significantly different ($p < 0.05$) from 0.6667 and their iron content may not be explained simply as a consequence of surface adsorption. Only in *S. christianiensis* (March)

was the power function not significantly different from 1.00 (table 3) indicating that iron concentration might be constant and independent of size. This, however, may be a spurious consequence of small sample size and correspondingly wide fiducial limits.

Since the iron concentration in different species is variously influenced by mass or surface area, mean values cannot legitimately be compared. For this reason, table 4 indicates only the ranges of body dry masses and iron concentrations. Iron concentration ranges have been quoted deliberately in descending order (cf. dry masses) to emphasize the generally higher concentrations of smaller animals, but it should not be assumed that the figure for Fe concentration first quoted relates to the lowest dry mass in any species.

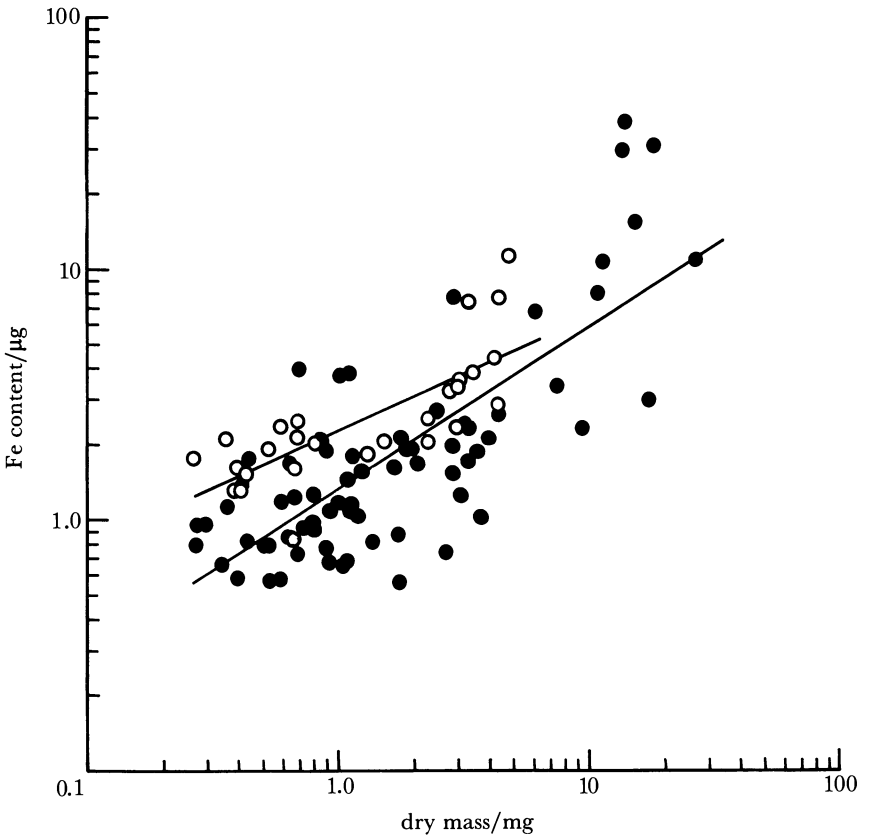


FIGURE 28. The relation between body iron content (in micrograms) and body dry mass (in milligrams) in *Stegocephaloides christianiensis* (open circles) and in all other marine amphipods combined (except *H. galba* from *Cyanea*, see text) (closed circles). Regression equations are given in text.

Iron levels in *S. christianiensis* were high, but not vastly higher, than many of the other species investigated (figure 28, table 4). Were *S. christianiensis* not to excrete Fe however, its body burden would presumably be very much elevated. The evidence therefore suggests that *S. christianiensis* may be regulating its tissue iron concentration at a level akin to the amphipod norm. Also implied therefore is acquisition of iron from a source unique to *S. christianiensis*; the most likely one being diet.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 4. Iron concentration in amphipod crustaceans from the Firth of Clyde

species	number of observations	date	dry mass range mg	range of Fe concentration per dry mass $\mu g\ g^{-1}$
Jassa falcata	7	May 1982	0.425–1.025	3688–845
Amphithoe rubricata	4	May 1982	0.435–17.275	3844–172
Microdeutopus versiculatus	2	May 1982	0.675–0.800	1822–1563
Apherusa jurinei	2	May 1982	0.510–2.075	1559–819
Hyale nilssoni	7	March 1982	0.932–1.935	1350–314
	9	May 1982	0.750–3.300	2227–276
Epimeria cornigera	4	March 1982	0.588–3.993	1176–528
	2	May 1982	0.800–7.475	1200–449
‡ *Gammarus duebeni*	10	May 1982	0.625–11.090	1609–97
† *G. pulex*	14	May 1982	4.710–17.075	1445–257
Bathyporeia pilosa	6	March 1982	0.270–0.395	3519–1494
Ampelisca macrocephala	7	March 1982	2.865–3.690	2616–276
Eusirus longipes	1	March 1982	1.143	962
Melphidipella macra	1	March 1982	1.658	983
Maera loveni	1	March 1982	26.620	379
Orchestia gammarellus	6	March 1982	10.830–18.070	2736–729
Caprella acanthifera	6	May 1982	0.675–1.390	5657–590
R *Hyperia galba*	5	March 1982	1.105–6.150	3484–598
C *Hyperia galba*	64	Sept. 1982	0.500–17.000	2467–74
Stegocephaloides christianiensis	27	Jan. & March 1982	0.265–4.791	6604–658

† Freshwater species; ‡ brackish water species; C, from *Cyanea*; R, from *Rhizostoma*. See text for explanation of reversed (descending) data on Fe concentration ranges.

6. The mouthparts of *Stegocephaloides christianiensis*

The mouthparts of stegocephalids are obscured from lateral view by the elongate anterior coxae (figure 29, plate 6). They project below the head in a characteristic conical bundle (Barnard 1969; our figure 30). This mouthpart bundle is strongly cuticularized: the base of the mandible, in particular, being reinforced with a honeycomb surface ornamentation (figure 30). In the animal at rest, the head is directed ventrally, thrusting the mouthpart cone down against the surface upon which the animal is resting. The anteriorly directed mandibular incisors and maxillae converge together on a small area of feeding surface (figure 31), with the large maxillipedal outer plate (figure 32) forming a posterior shield (retaining food particles?). Evidence will be presented later in support of the view that *S. christianiensis* feeds on the soft tissues of cnidarians. The large coxal plates probably serve as stabilizers steadying the animal during feeding, their lateral adduction perhaps serving to pinch together a ridge of tissue for the mouthparts to bite (a feature facilitated by the bilobed upper lip? figure 31). The slender peraeopods are well equipped to act as securing, grappling hooks and the feeble gnathopods may play only a limited role in feeding (although direct observations have proved impossible owing to the small size of the animals and the curtaining effect of the coxal plates).

The mandibles of stegocephalids are notably reduced, having lost both mandibular palp and triturating molar during evolution. This condition is approached elsewhere in the Gammaridea, only in the families Phliantidae, Eophliantidae and Prophliantidae. It is interesting that the eophliantids' mode of life is one of burrowing through relatively soft tissue (algae). The incisor process of the stegocephalid mandible, however, is well developed and strongly toothed, forming an efficient cutting edge (figure 33). The implication, on functional morphological grounds

alone, would be that *S. christianiensis* bites and swallows soft food, without needing to macerate it against molar grinding surfaces. The lower lip of stegocephalids lacks inner lobes (again a derivative condition). The apex of the outer lobe is drawn out into a papilla (figure 34) of unknown function (salivary duct? note Elofsson *et al.* 1978), and the inner face of the outer lobe is clad with inwardly recurving setules, which no doubt assist in gripping food particles.

7. The food of *Stegocepaloides christianiensis*

The expulsion of ferritin crystals by *S. christianiensis* and the comparable body iron burden of this species compared with other amphipods (see above) suggest a regulatory mechanism necessitated by excessive iron consumption. Such is the lot of many sanguivorous invertebrates, for example, leeches and trematodes (Jennings & Van der Lande 1967; Jennings 1968). A blood diet in those species with intracellular digestion involves degradation within the gastrodermis of haemoglobin into insoluble haematin granules before their expulsion via the gut lumen. The biting mouthparts of stegocephalids, plus the magenta-coloured, gluey-textured fresh stomach contents of *S. christianiensis* led to initial speculation that these amphipods too might be blood feeders.

Various lines of evidence, however, later shed doubt on this view. Thus *S. christianiensis* has been taken (1 October 1981) from the gut of the small goby (*Pomatoschistus norvegicus* (Collett)) of Kilchattan Bay, Isle of Bute, indicating a role as fish prey rather than fish attacker. The possibility that *S. christianiensis* might suck the blood or body fluids of annelids was also considered in the light of the presence of iron-based haemoglobin and chlorocruorin in certain worms. Four starved amphipods which had been kept in filtered seawater (4 °C) for 10 d, were introduced into an aquarium which contained a clump of fanworms (*Sabella pavonina* Savigny) and their reactions noted. The amphipods swam around haphazardly, intermittently resting on the bottom. No directional response to the worms was apparent. The amphipods made no attempts to feed that could be seen, but merely used the tubes as occasional, convenient 'footholds'. A similarly starved *S. christianiensis* showed no reaction to the presence of a live *Sabella* deprived of its tube. During the course of a different investigation, it was noted that *S. christianiensis* was not attracted to traps baited with dead crabs (*Cancer*, *Carcinus*), dead whitefish (*Gadus* spp.) or artificial bait (tinned cat food) left for 24 h at 80 m over mud off Kilchattan Bay (20–21 October 1981) when these traps yielded an abundance of scavenging lysianassid amphipods (*Scopelocheirus hopei* (Costa)), and the same ground yielded an abundance of *Stegocephaloides christianiensis* in D-net hauls. The lack of eyes suggests that *S. christianiensis* relies on chemosensation for prey detection. Yet it is clearly not a generalist scavenger.

Known crustacean sanguivores, for example, gnathiid isopod praniza larvae and the copepod *Lernaeocera* were examined for evidence of gut Fe-crystals but none was found.

The stomach contents of 10 freshly collected *S. christianiensis* were examined in January and June 1982. Stomach contents in this species were always magenta coloured irrespective of animal size and the smallest investigated animal (3 mm in length) had Fe crystals in the gut caeca, suggesting that diet remains unchanged with age. The only identifiable items in the stomachs of *S. christianiensis* were sand grains, iron crystals and two basic types (long and oval) of cnidarian nematocysts (table 5, figures 35–37). Incidentally, it may be mentioned that one of the amphipods (number 10) examined in January had hundreds of small (most *ca.* 22 μm long, the biggest being *ca.* 45 μm, see figure 38*a*), unidentified ciliates in its body cavity. Finding

TABLE 5. THE DISTRIBUTION OF RECOGNIZABLE ITEMS IN THE STOMACH CONTENTS OF FRESHLY COLLECTED *STEGOCEPHALOIDES CHRISTIANIENSIS*

	animal number									
	1	2	3	4	5	6	7	8	9	10
January										
sand grains	+	+	+	+	+	+	+	+	+	+
long nematocysts	+	−	+	−	+	+	+	+	+	−
oval nematocysts	+	+	−	−	−	+	−	−	−	−
% with sand grains	100									
% with nematocysts	80 (70% long; 30% oval)									
June										
sand grains	−	−	+	+	+	+	+	+	+	+
long nematocysts	+	−	+	−	+	−	−	++	−	+
oval nematocysts	+	−	+	+	+	−	+	+	+	+
% with sand grains	80									
% with nematocysts	80 (50% long; 80% oval)									

S. christianiensis collected from about 60 m off Fintray Bay, Isle of Cumbrae, 7 January and 4 June 1982.

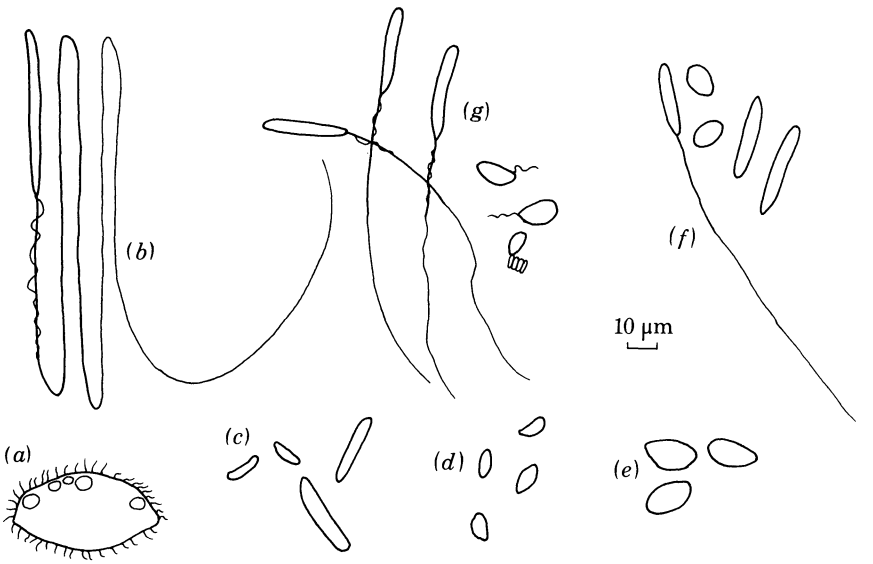


FIGURE 38. (*a*) Unidentified ciliate from body cavity of *S. christianiensis*, (*b–f*) nematocysts of common cnidarians from *Stegocephaloides* collecting grounds, drawn from simple squash preparations. (*b*) *Bolocera tuediae*, (*c*) *Pennatula phosphorea*, (*d*) *Hydractinia echinata*, (*e*) *Hydrallmania falcata* and (*f*) *Adamsia carciniopados*. (*g*) Nematocysts from squash preparations of stomach contents of *S. christianiensis*. Scale line, 10 µm.

nematocysts led to the final abandonment of investigations of possible sanguivory in favour of a predatory diet centred on cnidarians (many of which are soft in texture and red-brown in colour).

The nematocysts of common cnidarians from the *S. christianiensis* collecting grounds were examined in simple squash preparations of tentacles or column tissue (figure 38). Neither the anemone *Bolocera tuediae* (Johnston) nor the hydroid *Hydrallmania falcata* (L.) had nematocysts which matched those from *S. christianiensis* stomach contents. The small hydroid *Hydractinia echinata* (Fleming), which lives commensally on shells occupied by hermit crabs (*Pagurus bernhardus* (L.)), however, had nematocysts which approximated both in size and shape to the

oval nematocysts from *S. christianiensis* stomachs. Also similar, were the long (predominating type, cf. table 5) and oval nematocysts derived from the cloak anemone *Adamsia carciniopados* (Otto) (= *A. palliata*) (an associate of the hermit crab *P. prideauxi* (Leach)). The pinky-red, retractile acontia of *Adamsia* gave a Prussian blue reaction for iron. In addition, the long, straight and long, curved nematocysts from *S. christianiensis* stomachs (figures 36 and 37) matched in size those of the sea-pen *Pennatula phosphorea* L. Each of these cnidarians would be reliably available *in situ*. Each has some degree of red pigmentation (most pronounced in *Pennatula*) which could provide an alternative explanation for the soft, red 'mush' which is so characteristic of the fresh stomach contents of *S. christianiensis*. Exhaustive nematocyst cross-matching of all local cnidarians was impracticable. Further clues, however, were sought (i) from behavioural observations on live *S. christianiensis* (September 1982) and (ii) from analyses of the iron content both of selected cnidarians (total body and dissected tissues) and of known arthropod predators of cnidarians.

The common anemone from the *S. christianiensis* collecting grounds *Urticina eques* (Gosse), when put in an aquarium with three live *S. christianiensis*, ate them immediately they touched its tentacles and so may presumably be ruled out as a potential prey species. Three freshly collected *S. christianiensis* introduced into an aquarium containing a hermit crab (*Pagurus prideauxi*) with *Adamsia carciniopados*, showed no directional orientation towards the anemone. One amphipod, coming into accidental contact with the ventrally directed tentacle ring of the anemone, was immediately devoured. Amphipods coming into contact with the anemone's column did not cling to it (though they are capable of clinging onto the smooth walls of a plastic aquarium). One *S. christianiensis* deliberately placed on the *Adamsia* lodged there momentarily but did not grip the substratum and was dislodged by the first lurch of the hermit crab. Two freshly collected *S. christianiensis* were confined also in an aquarium with a sea-pen *Pennatula phosphorea* (without sediment). No immediate orientation was shown but after 1 h 45 min the larger amphipod came into apparently accidental contact with the top of the sea-pen. The amphipod moved around in an upright posture (that is, gripping the substratum) between the leaves, unhampered by adhesion. The amphipod was not disturbed by a small deliberate shake of the sea-pen. Intermittent observations showed that this contact was maintained for at least 4 h 30 min. The following morning, the amphipod was found separated from the *Pennatula*. It responded vigorously to stimulation and was clearly none the worse for its period in contact with the sea-pen. The stomach content of this amphipod was examined and nematocysts of a size and shape comparable with the small, elongate, curved nematocysts found in *Pennatula* (figure 38) were discovered, implying that feeding had been taking place. Sand grains and detritus are often found adhering to the mucoid secretions of *Pennatula* which could explain the occurrence of sand in *S. christianiensis* stomachs (table 5).

Table 6 itemizes the Fe levels in local cnidarians and in the deep water medusa *Atolla wyvillei* Haeckel (included since many stegocephalid species are bathypelagic). The sea spider *Pycnogonum littorale* (Ström) is a known predator of cnidarians (King 1974) and comparison may also be made with levels in *Hyperia galba* which consumes *Cyanea* tissues (Dahl 1959).

Iron levels in most cnidarians studied varied between 100 and 500 $\mu g\ g^{-1}$ dry mass. Certain of the species, however, that were selected as possible prey organisms on the basis of nematocyst morphology, had Fe concentrations an order of magnitude higher, for example, *Pennatula* and *Adamsia*. Unfortunately, we were unable to take effective samples of the mat-like hydroid *Hydractinia* for chemical analysis. The high Fe levels of whole *Adamsia* must be attributed in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 6. IRON CONCENTRATIONS IN CNIDARIANS AND KNOWN CNIDARIAN PREDATORS

species	number of observations	date	dry mass range mg	range of Fe concentration per dry mass $\mu g\ g^{-1}$
Cnidaria				
Urticina eques (tentacles)	5	Sept. 1982	3.9–19.4	433–163
Bolocera tuediae (tentacles)	3	Sept. 1982	5.6–7.3	552–241
Actinia equina (whole)	5	Sept. 1982	377.0–609.3	147–90
Stomphia coccinea (whole)	6	Sept. 1982	274.4–541.1	250–77
Adamsia carciniopados (whole)	5	Sept. 1982	241.7–404.4	2269–662
A. carciniopados (column)	2	Sept. 1982	2.8–12.6	657–238
A. carciniopados (tentacles)	1	Sept. 1982	14.3	292
A. carciniopados (acontia)	5	Sept. 1982	1.0–9.6	1010–228
Metridium senile (whole)	6	Sept. 1982	75.0–266.3	270–120
Alcyonium digitatum (white)	4	Sept. 1982	938.6–1536.0	152–62
A. digitatum (orange)	5	Sept. 1982	344.8–1544.5	196–90
Pennatula phosphorea (whole)	5	Sept. 1982	57.0–1452.0	3158–200
P. phosphorea (soft tissue, entire)	1	Feb. 1983	345.7	878
P. phosphorea (soft tissue, rachis)	1	Feb. 1983	875.9	1055
P. phosphorea (soft tissue, peduncle)	1	Feb. 1983	335.0	717
Virgularia mirabilis (whole)	2	Feb. 1983	120.97–174.4	917–411
V. mirabilis (soft tissue, entire)	2	Feb. 1983	127.22–140.96	2186–1213
Nemertesia antennina	5	Sept. 1982	15.6–94.5	5827–998
Cyanea capillata (bell)	1	Sept. 1982	796.6	48.3
C. capillata (tentacles)	2	Sept. 1982	525.4–1108.1	151.3–53
Atolla wyvillei (whole)	2	April 1982	1689.6–9929.1	9.2–5.8
Pycnogonida				
Pycnogonum littorale	7	May 1982	5.05–8.15	1886–630
Crustacea–Amphipoda				
Hyperia galba (from *Cyanea*)	64	Sept. 1982	0.5–17.0	2467–74

Samples collected from the Firth of Clyde, excepting *Atolla* from Gulf of Guinea (depth 1000 m).

large measure to levels of iron in the horny, basal membrane (which the anemone secretes as a 'porch' over its associated hermit crab), since excised column tissue gave lower (though still high) Fe concentrations. The nematocyst-laden, discharged acontia of *Adamsia* were particularly rich in Fe. Removal of the calcareous axial rods from the two sea-pen species (*Pennatula* and *Virgularia*) elevated the iron concentrations, especially in the rachis and leaves of *Pennatula*, suggesting that Fe may be localized in the soft tissues. Small skeletal spicules, however, remain in the tissue after this treatment and since in *Pennatula* these are red in colour, some iron may still be bound skeletally. The high Fe levels in *Pennatula*, *Virgularia* and *Nemertesia* may also be influenced by adsorption onto their relatively large surfaces (note also *Pycnogonum* and *Caprella*; table 4). By contrast, the Fe levels in *Atolla* were extremely low. Clearly, further work is necessary to establish the form and availability of iron in cnidarian soft tissue.

Iron concentrations in *Pycnogonum* and *Hyperia* were higher than in many of the cnidarians analysed. This may be attributable to bioaccumulation, but again could also (or partially) be influenced by their small body size and relatively large surface areas.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

8. Discussion

Hepatopancreatic iron granules have been reported in a variety of decapod crustaceans (reviewed by Gibson & Barker 1979) and Rainbow & Walker (1977) found ferric iron granules in posterior midgut cells of the cirripedes *Lepas anatifera*, *Pollicipes mitella* and *Balanus hameri*. Less widely known, however, is their occurrence within the Peracarida (particularly Isopoda). They occur in the woodlouse *Porcellio scaber* (Hryniewiecka-Szyfter 1971–2), in the deep sea isopod *Bathynomus giganteus* (Steeves 1968) and we have observed a histochemical tissue reaction for iron and irregular, Fe-staining granules in the gut caeca cells of the scavenging marine isopod *Cirolana borealis* Liljeborg. Jones *et al.* (1969) described proteinaceous, iron-rich crystals (0.1–30 μm in size) in the caeca of another cirolanid isopod *Eurydice pulchra* and regular, octahedral shaped crystals of an iron–protein composition were reported in the gribble *Limnoria lignorum* by Fahrenbach (1959) and by Strunk (1959). Hassall & Jennings (1975) found organically bound iron occurring as cytoplasmic granules in the midgut caeca of the woodlouse *Philoscia muscorum*. Largely unpublished observations by Donadey (1973, but see also 1968, 1972), on a wide range of isopod species, confirm the iron–protein composition of these cellular inclusions. Interestingly, the terrestrial genera he examined, that is, *Porcellio*, *Oniscus*, *Armadillio* and *Armadillidium* did not possess crystals, neither did the freshwater *Asellus*. Only the benthic isopods from fully marine habitats contained gut crystals at some stage of the intermoult period, namely, *Idotea balthica basteri*, *I. granulosa*, *I. emarginata* (see also Beecher-Moore 1959), *I. hectica*, *Eurydice affinis*, *E. pulchra*, *Sphaeroma serratum*, *Cymodoce truncata*, *C. spinosa*, *Dynamene bidentata* and *Limnoria lignorum*.

Also presently germane is Donadey's (1973) observation (i) that crystals were not found in those parasitic isopods feeding on blood in the buccal cavity of fishes (*Nerocila bivittata*, *Anilocra physodes*, *Meinertia oestroides*) and (ii) that crystal presence varied according to the stage in the animals' moult cycle (see also Donadey 1968, 1972), with animals at stage D_0 (just preceding the moult, when feeding ceases) having the maximum complement. Fahrenbach (1959) noted that crystals were not consistently present in *Limnoria*. We have examined *Limnoria* ourselves and failed to find crystals (although the caecum tissue gave a strong histochemical reaction for iron). Variations in the moult stage of individuals may help explain such inconsistencies. Within the Amphipoda, such crystals are presently known only from members of the family Stegocephalidae (Moore 1979 and present report; reviewed by Icely 1981).

The common properties of isopod and amphipod crystals deserve reiteration. Crystals are octahedral in shape, yellow-brown in colour and up to 30 μm in size. So far, published speculation as to their nature has been restrained. At magnifications in excess of 50000×, however, crystals in all isopods (Strunk 1959; Donadey 1973) and in the amphipod *Stegocephaloides christianiensis* (above) are seen to conform in ultrastructure. That is, they are built as a regular lattice made up of spherical subunits approximately 6 nm in diameter at 9 nm centres. This configuration with characteristically spaced electron diffraction rings (as yet untested in Isopoda) is consistent with the known crystalline properties of ferritin, whether of vertebrate (Crichton 1973; Harrison 1977, 1981; Richter 1978) or invertebrate origin (Towe *et al.* 1963; Heneine *et al.* 1969; Nardi *et al.* 1971). On these bases we propose that the isopod (see Strunk 1959) and amphipod Fe-protein crystals are both made up of ferritin. Other workers have recently reported ferritin-like proteins in crustaceans. Guary & Négrel (1980) found that iron was associated in the hepatopancreas of the crab *Cancer pagurus* with a protein of apparent

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

molecular mass 450000 Da which they thought could be crustacean ferritin and Yamauchi *et al.* (1981) purified an iron-bound protein (molecular mass 520000 Da) from extracts of whole *Daphnia magna* which they identified as ferritin-like.

Ferritin is a substance of extraordinarily wide occurrence in living organisms. In animals it is particularly associated with the most metabolically active tissues, for example, digestive glands, kidneys, gonads, muscles. It is generally regarded as having a storage function (Haggis 1965; Harrison 1977). Free iron is toxic and its sequestration within the apoferritin protein shell serves as a detoxification mechanism (Harrison 1977). Reactivation, however, is not precluded (Heneine *et al.* 1969; Coombs & George 1978). The mobilization of iron atoms is necessary at metabolically expensive sites to facilitate biosynthesis or to catalyse enzymic redox reactions (Steeves 1969). Digestive glands perform diverse metabolic functions (digestion, absorption, secretion, storage), so that it is not surprising that ferritin recurs throughout the animal kingdom in such organs or at such sites of iron deposition as the molluscan radula (Runham *et al.* 1969). Thus in the chitons *Acanthochites fascicularis* and *Cryptochiton stelleri* a large reserve of iron is held (at any rate in the latter) as ferritin, in the dorsal radular epithelium (Gabe & Prenant 1948; Towe *et al.* 1963). The potential for remobilization could explain Donadey's (1973) observations that in isopods the occurrence of iron-rich crystals varied with the moult stage. While specific experiments on moulting in *Stegocephaloides christianiensis* have yet to be accomplished, it is worth noting that all animals yet examined (of either sex or of whatever size, nutritional state, etc.) have proved to possess crystals. Perhaps there is no variation with moult stage in *S. christianiensis* or possibly any such variation takes place against such a high 'background' level as not to be noticed qualitatively.

The key distinction between *S. christianiensis* and the marine benthic isopods with crystals and other marine amphipods without (even sediment ingesters), is the particularly high iron content in its presumed food. In disparate organisms, ferritin biosynthesis seems to be induced by the supply of iron from the environment, for example, in plants (Seckbach 1968) and in rat liver (Drysdale & Munro 1966). It is interesting, however, that Fahrenbach (1959) found no significantly higher iron content in the isopod *Limnoria* fed wood impregnated with iron salts compared with controls. Unfortunately though, neither actual iron levels in the woods used nor the quantities of stored iron in the gribbles, were reported. He decided that it was most likely that *Limnoria* was incapable of excreting iron and that instead, it stored Fe in the form of crystals (ferritin, see above) when the cytoplasmic iron content rose to a critical (unspecified) level. So far as can be ascertained, however, no examination of faeces was made. As an aside it may be noted that we have not obtained any tissue Fe reaction or seen any crystals in the gut caeca of the amphipod *Chelura terebrans* Philippi, which shares the lignivorous habits (indeed sometimes the same burrows) as *Limnoria*.

Excretion of iron as ferritin has been demonstrated here in *Stegocephaloides christianiensis*. A role for ferritin in iron excretion is novel. A mechanism involving successive translocations and eventual elimination of Fe, presented as iron hydroxide, has been proposed by George *et al.* (1976) in the bivalve *Mytilus edulis*, with a major proportion of excreted iron being laid down in the byssal threads. Iron presented as ferritin, however, is immobilized and not excreted by *M. edulis* (George *et al.* 1975). These authors expressed concern about the implications for the food chain if toxic metals were to be immobilized by sequestration into metalloprotein complexes, thus blocking normal excretory processes. Our findings suggest that, in amphipod crustaceans at any rate, even were the iron moiety to be tightly chelated with protein, the whole

metalloprotein complex may be susceptible to expulsion with the removal of ageing cells. Similar propositions have recently been made by Icely & Nott (1980) in connection with the excretion of copper granules from the gut caeca of another amphipod *Corophium volutator* and by Prosi *et al.* (1983) working on lead in the isopod *Porcellio scaber*.

Many workers have described the ultrastructure of the midgut and hepatopancreas in a variety of crustacean groups, for example, barnacles (Rainbow & Walker 1977), copepods (Arnaud *et al.* 1978), peracarids (Jones *et al.* 1969; Moritz *et al.* 1973; Hassall & Jennings 1975; Schultz 1975) and decapods (Miyawaki & Tanoue 1962; Gibson & Barker 1979). Complex schemes of cell notation have been developed which centre on the belief that different cell types have different functions (absorption, secretion, etc.). Jones *et al.* (1969), Vernon *et al.* (1974) and Hassall & Jennings (1975), however, favoured the view that the structural cellular heterogeneity apparent in the caeca of isopods was the result of individual cells being arrested at different stages in a universal cycle of cell growth. According to Hassall & Jennings (1975) juvenile cells are absorptive and take up material from the caecal lumen. Once mature they assume a predominantly secretory function, producing digestive enzymes. The present investigation has not sought specifically to clarify this issue with respect to *S. christianiensis*, but it is of interest that Martin (1964), investigating the amphipod *Echinogammarus* (= *Marinogammarus*) *obtusatus*, considered that there was no clear demarcation of function between the so-called secretory and reserve cells (see also Schmitz 1967). More recently, Icely (1981) in a thesis on the amphipod *Corophium volutator*, also considered that there were insufficient differences in mature cells from the ventral caeca to warrant their distinction as R and F cells. He called them R/F cells, describing them as being derived from embryonic E cells at the distal, blind end of the tubule and capable of further differentiation into B cells. We would concur with his structural categorization for *S. christianiensis*.

It is only proximal to caecal zone I (*sensu* Schultz 1975) that ferritin crystals are laid down in R/F cells of *S. christianiensis*. Moore (1979, figure 1*b*) showed a longitudinal section of zones II (with initial development of small crystals) and III (with larger crystals). Crystal size thus reflects cell age and larger crystals would be anticipated in cells of the proximal region if this was primarily the site of absorption and storage. Interestingly, Prosi *et al.* (1983) found that lead was localized only within the proximal region of the midgut caeca in the woodlouse *Porcellio*. Lipid appears to be the primary storage product in *S. christianiensis* R/F cells, but glycogen (cf. Schultz 1975) was also identified (see also Mabillot 1955; Martin 1965; Icely 1981). Of singular interest are Icely's (1981) observations on *Corophium volutator* fed ferritin as a tracer for intracellular particulate uptake. Ferritin was not readily absorbed by R/F cells in the ventral caeca, except where the cells were ageing and the microvilli breaking down; it was more readily absorbed into the apical complex of B cells. Uptake of iron saccharate by R/F cells of *Echinogammarus obtusatus*, however, was described by Martin (1964). The chemical form in which *S. christianiensis* encounters Fe is not known so uptake route(s) cannot yet be proposed.

Strunk (1959) and Jones *et al.* (1969) described ferritin (our identification) crystal formation in association with membrane complexes (Golgi) in the isopods *Limnoria lignorum* and *Eurydice pulchra* respectively. According to Strunk, after a crystal has been formed, the membranes on which synthesis occurred disappear, leaving even numbers of double parallel rows of particles without supporting membranes. These particles subsequently orient themselves in a hexagonal, close-packed pattern. In *S. christianiensis*, crystal precursors may be synthesized in the densely

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

granular rough endoplasmic reticulum which breaks down at the crystals' edges or perhaps on free polyribosomes (see Richter 1978).

Incidental to the issue of the iron crystals, we referred earlier to calcium-rich granules in stegocephalid caecum cells. Briefly, calcium granules in invertebrate tissues have been recognized recently as of two types, carbonate and pyrophosphate (Simkiss 1981). The carbonate variety represents a system which, through dissolution of the granules, can operate in fluid buffering and calcium balance (Mason & Nott 1981) and in amphipods would be likely to be involved in calcium mobilization during the moult cycle (Graf 1977). The pyrophosphate granules, on the other hand, apparently represent a system for detoxifying certain cations, since they form extremely insoluble salts with a wide variety of metal ions (Simkiss 1980; Mason & Nott 1981; Howard *et al.* 1981; Simkiss *et al.* 1982). Unfortunately, definitive characterization of the calcium granules presently encountered was beyond the limits of the analytical methods used. Clarification of their composition would be of great interest, however, since most previous work on Ca granules has centred on gastropod molluscs.

An iron challenge is met in different ways by different organisms. The dugong (*Dugong dugon*) tolerates liver iron levels as high as 82363 μg g^{-1} dry mass, laying down iron (derived from its high Fe content seagrass food) as haemosiderin in quantities which tend to increase with age (Denton *et al.* 1980). Sanguivorous helminths with intracellular digestion may lay down haematin (the haemoglobin breakdown product) as granules in the gastrodermis (Jennings 1968; Jennings & Van der Lande 1967) and then void them into the gut lumen. In the Echinodermata, on the other hand, Buchanan *et al.* (1980) have reported massive deposition of iron (as granules of ferric phosphate) in the connective tissue layer of the large intestines of the spatangoid urchins *Brissopsis lyrifera*, *Echinocardium cordatum*, *E. flavescens* and *E. pennatifidum*. Iron apparently accumulates there with age as an inevitable consequence of the ingestion of soluble Fe(II)-phosphate species during feeding. The sea urchins do not void the iron into the gut lumen.

Levels of iron as high as 173000 μg g^{-1} dry mass were recorded in the large intestine of *Brissopsis* by Buchanan *et al.* (1980). Unfortunately, data on crustaceans are few and relate mainly to decapods (White 1982). Levels in shrimps (*Palaemon elegans* Rathke) of 15–590 μg g^{-1} dry mass were found by White, compared with data in the literature for crabs (205), lobsters (20), euphausids (54–570), copepods (55–382) and barnacles (475). Prosi *et al.* (1983) reported levels of Fe between 136.5 and 995 μg g^{-1} dry mass in the woodlouse *Porcellio scaber* in Germany. For amphipods, Bender (1975) found comparable levels of Fe between 50 and 120 μg g^{-1} dry mass in the supralittoral beach hopper *Orchestoidea corniculata* and 30–120 μg g^{-1} in the related *O. californiana*. Bohn & McElroy (1976) noted 87 μg g^{-1} in unspecified Canadian Lysianassidae. Icely & Nott (1980) reported 325 ± 94 μg g^{-1} dry mass in *Corophium volutator* from Dulas Bay, Anglesey with significantly higher (494 ± 94 μg g^{-1}) levels in the same species from the more turbid Menai Strait. Our own observations, on a wide range of species from the Firth of Clyde (table 4) reveal levels between 172 and 6604 μg g^{-1} dry mass, but comparisons must be drawn with caution owing to the effects of body size noted earlier. Fowler (1974) and Bender (1975) reported a decrease in iron concentration with size in amphipods *inter alia*, which our findings reflect in depth.

Sadly, the biology of the chiefly bathypelagic family Stegocephalidae remains largely obscure. Enequist (1949) reported finding clay particles inside freshly caught *Stegocephalus inflatus* and *Phippsiella similis* but could not induce detritus-eating in an aquarium. He noted

that the animals showed no tendency to burrow. Stegocephalids are fast swimmers (Enequist 1949; Moore 1979) and most likely all are pelagic or suprabenthic predatory grazers (see Barnard 1961, 1969). Dr W. Vader (personal communication) reported often finding *Stegocephalus* with 'sea trees' (*Paragorgea* (−Alcyonaria)) and the like and *Phippsiella* on large sponges of the genus *Geodia* in Norwegian seas. *Phippsia gibbosa* apparently lives on *Lophelia* corals, while *Andaniella pectinata* has been found repeatedly in ascidians. Dr Vader knew of no associations involving *Andaniexis* or *Andaniopsis*, which he regarded simply as common hyperbenthic species (*Andaniexis spongicola* Pirlot, however, is known as an inquiline in hexactinellid sponges (Pirlot 1933)); *Stegocephaloides christianiensis*, in Vader's experience, was usually obtained singly or a few together on shell, gravel or Modiola-bottoms. Barnard (1961) described the stomach contents of *Parandania boecki* as a spongy mass (presently confirmed) composed of finely particulate matter and three kinds of empty cell 'tests', one kind spherical, another oval, the third a clavate sac (nematocysts?). Indeed, he speculated that *P. boecki* may feed on coelenterates or salps. Perhaps the relatively huge stomach in *P. boecki* can be regarded as an adaptation to an uncertain food supply in the bathyal realm. In *Stegocephaloides attingens*, however, Barnard (1961) found that 50% of the stomach contents consisted of mineral particles, the remainder being finely particulate organic matter.

Present evidence, although incomplete, thus suggests that there may be a link between stegocephalid species which possess ferritin crystals in the gut caeca and a diet of cnidarians.

Amphipods are not unknown as associates of Anthozoa, for example, *Metopa solsbergi* on *Metridium senile* (Elmhirst 1925), *Abludomelita obtusata* on *Anemonia viridis* (= *A. sulcata*) (Hartnoll 1971) and coral reefs characteristically support an abundance of amphipods. Although *Metridium* may be depauperate in nematocysts, *Anemonia* maintains an armoury worthy of human (let alone amphipod) respect. Similarly, *Hyperia galba* associates with the badly stinging scyphozoan *Cyanea capillata* with impunity (Dahl 1959). Paradoxically then, the potency of nematocyst defences may be a poor guide to the use of a cnidarian as an amphipod host. Neither should the small size of *Stegocephaloides christianiensis* prejudice association with a large host, if the *Metopa–Metridium* duo is anything to go by. Perhaps *S. christianiensis* can escape nematocyst entanglement by virtue of its highly smooth cuticle (figure 29; Bousfield 1978)?

Though the general hypothesis that arthropods feeding on cnidarians have elevated iron concentrations cannot be established without further investigation, neither is it refuted by present evidence. Of interest would be data on iron levels in other consumers of cnidarians, for example, nudibranch molluscs. Data on the iron concentration of very few cnidarians have hitherto been published. Riley & Segar (1970) reported levels of 730 μg g^{-1} dry mass in *Tealia felina* and 250 in *Alcyonium digitatum* from the Irish Sea. These figures are in broad agreement with our own findings (table 6) that most Clyde cnidarians have Fe concentrations between 100 and 500 μg g^{-1} dry mass although this may be elevated by an order of magnitude in certain species or in particular tissues. Given our discovery that *Adamsia* acontia are rich in Fe, it is interesting to note that Mariscal (1980), using X-ray microanalysis, has found iron in the nematocyst threads of the gymnoblast hydroid *Pennaria tiarella* (but not in the capsule). These findings beg the question of the role(s) of iron in the Cnidaria. It is known, however, that the horny matrix within the calcareous skeleton of corals has a special affinity for iron (Fox & Pantin 1944; Kawaguti & Sakumoto 1954) but little is known about tissue iron concentration though a variety of pyrrolic haematins and bile pigments (MacMunn 1885, 1886; Lederer 1940; Fox & Pantin 1944), as well as cytochromes (Goodwin 1968), have been reported in

cnidarian soft tissues. That many iron-rich compounds (both pigmented and non-pigmented) exist in cnidarian tissues, provides insight into the likely source of the iron challenge faced by *Stegocephaloides christianiensis* and other stegocephalid cnidarian grazers.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Abbreviations used on figures

a.p.	apical papilla
b.m.	basement membrane
c.1	first coxal plate
Ca g.	calcium granule
cyt.	cytoplasm
Fe c.	iron crystal
g.	granule
G.b.	Golgi body
h.o.	honeycomb ornamentation
i.p.	incisor process of mandible
l.g.	lipid globule
l.m.	long microvilli
l.mnd.	left mandible
l.mx.1	first maxilla (left)
l.n.	long nematocyst
m.	mitochondrion
mx.p.	maxillary palp
mxp.o.p.	maxillipedal outer plate
mxp.p.	maxillipedal palp
n.	nucleus
o.n.	oval nematocyst
r.e.r.	rough endoplasmic reticulum
r.mnd.	right mandible
r.mx.1	first maxilla (right)
r.mx.2	second maxilla (right)
s.g.	sand grain
s.m.	spiral muscle
st.m.	stubby microvilli
u.l.	upper lip
v.	vesicle

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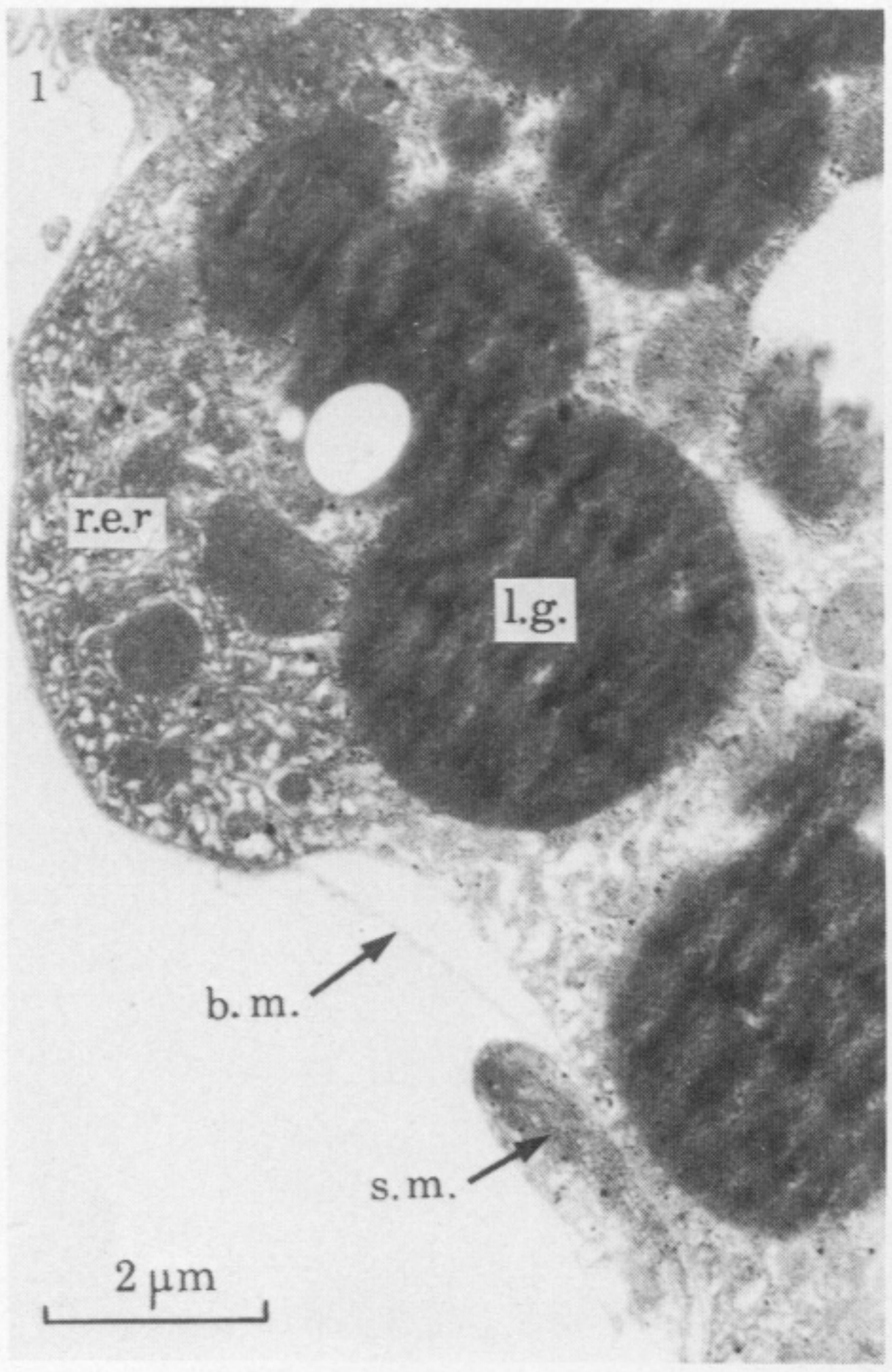
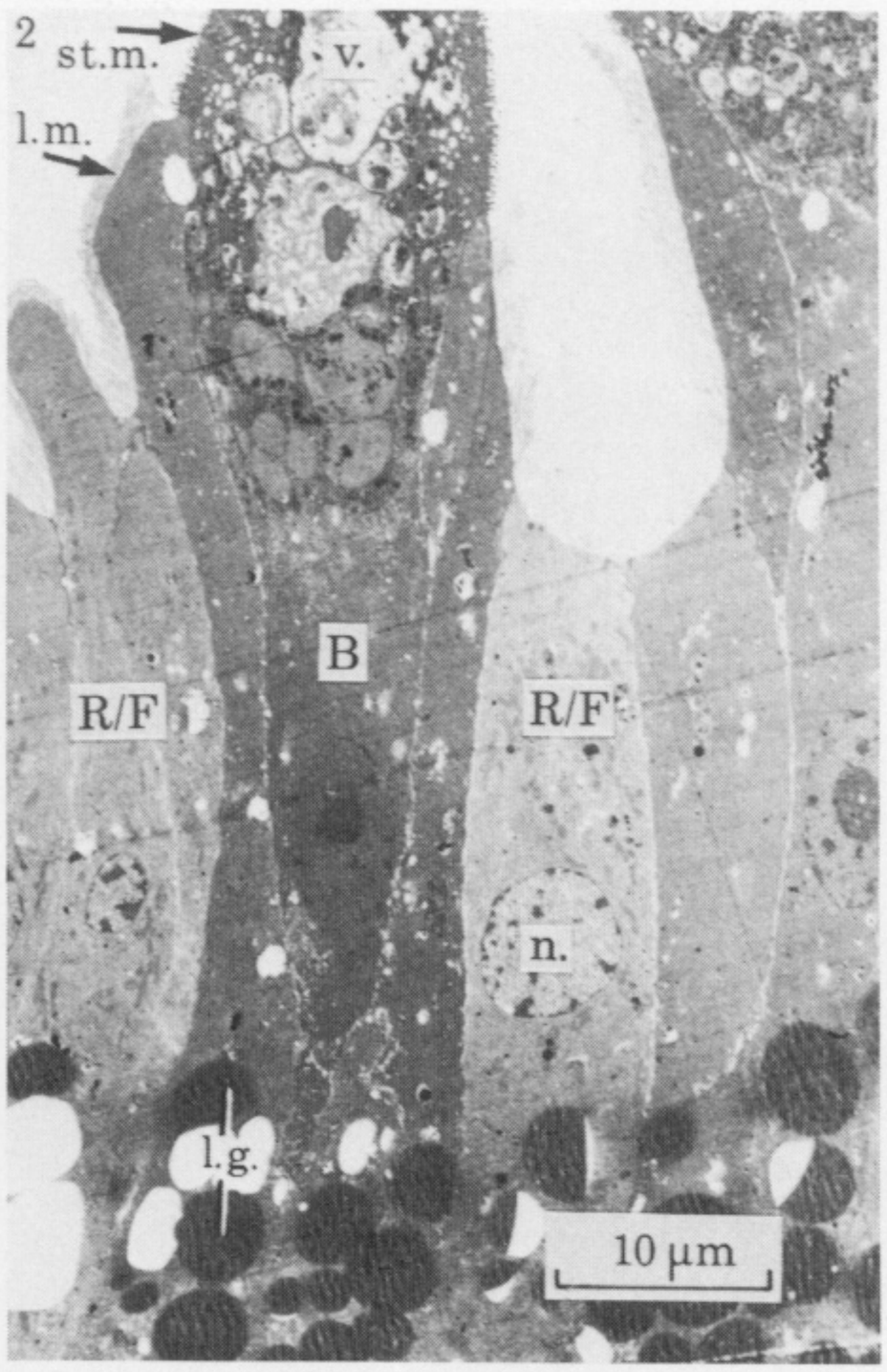
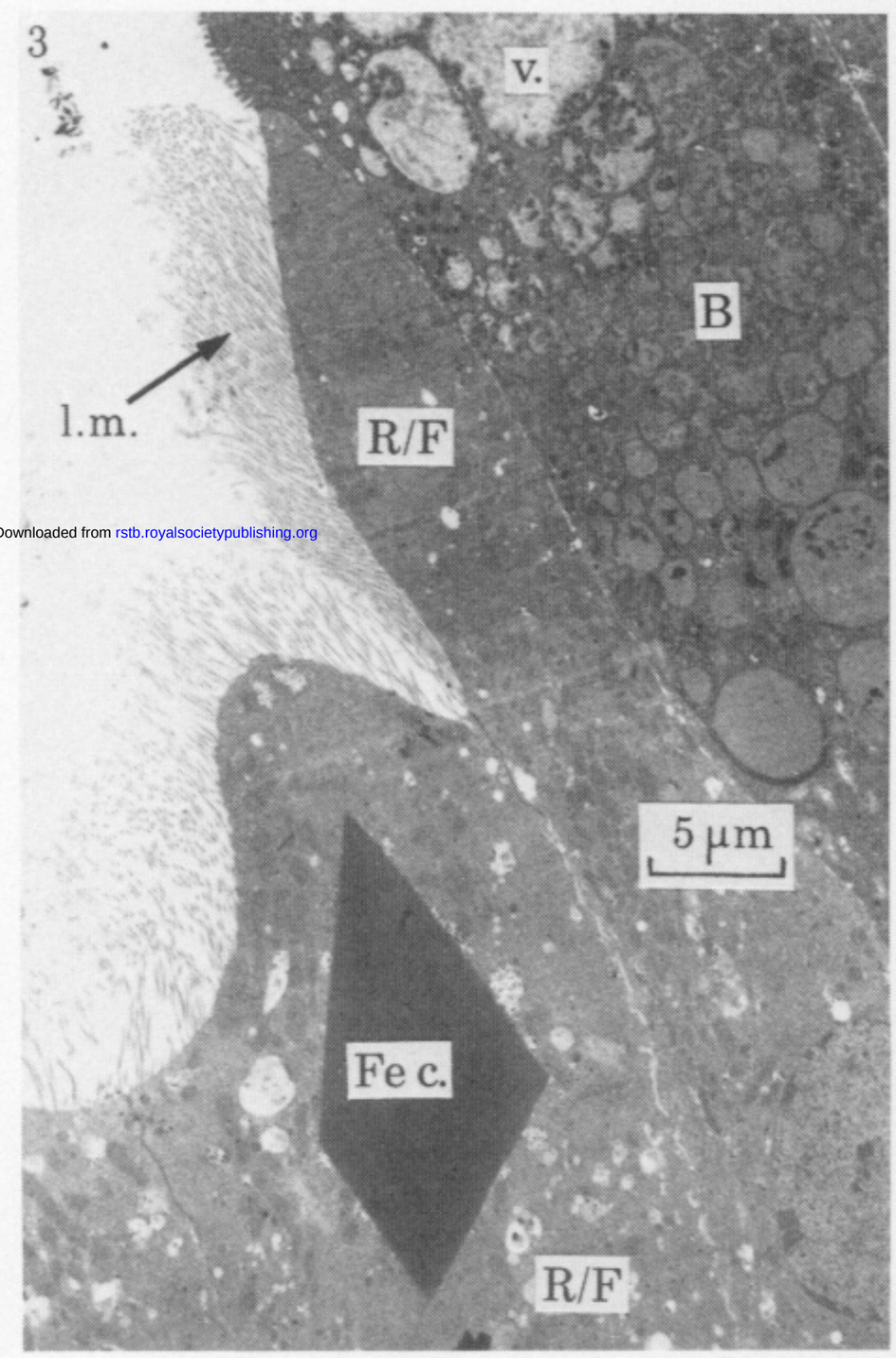






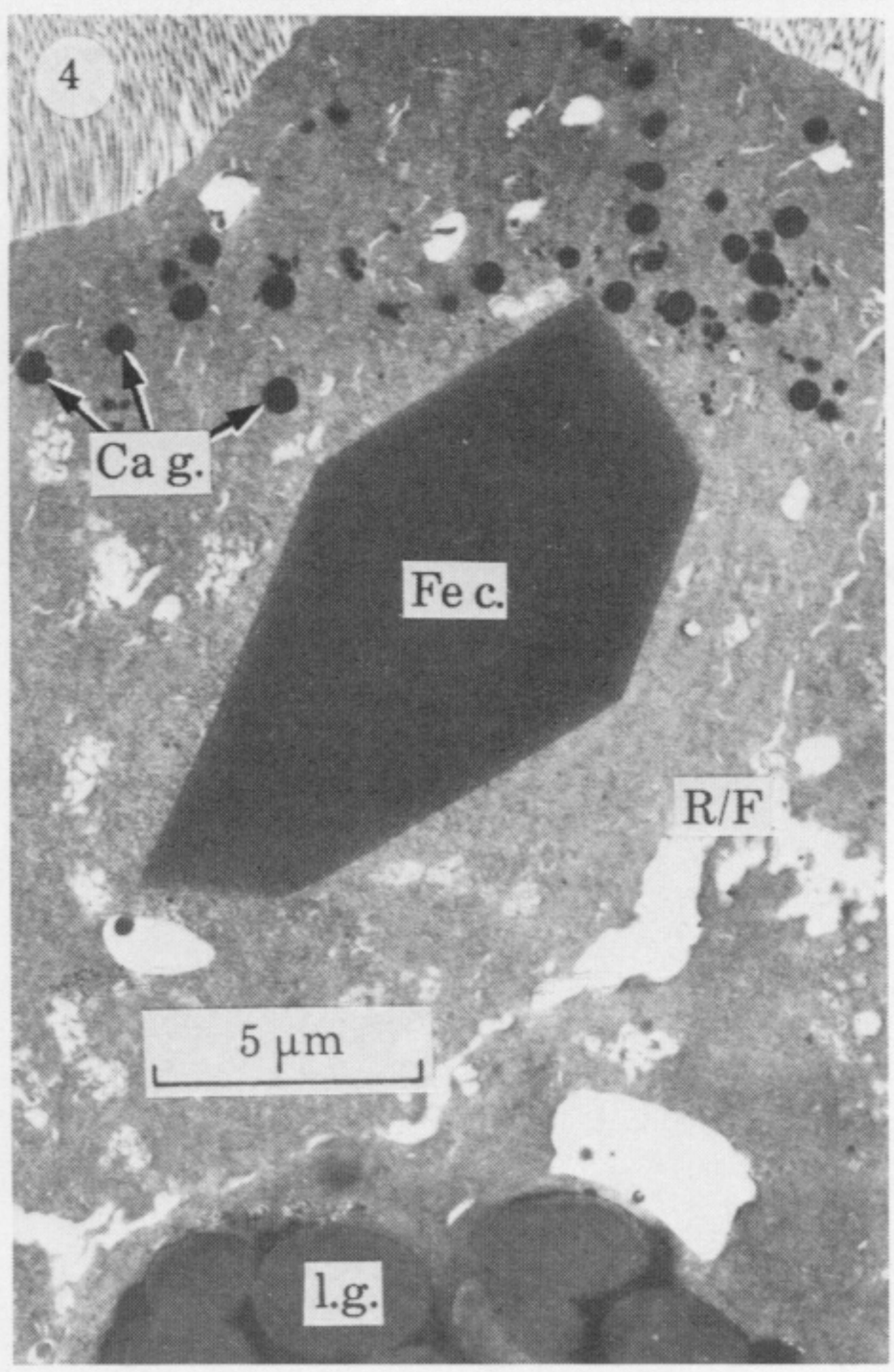
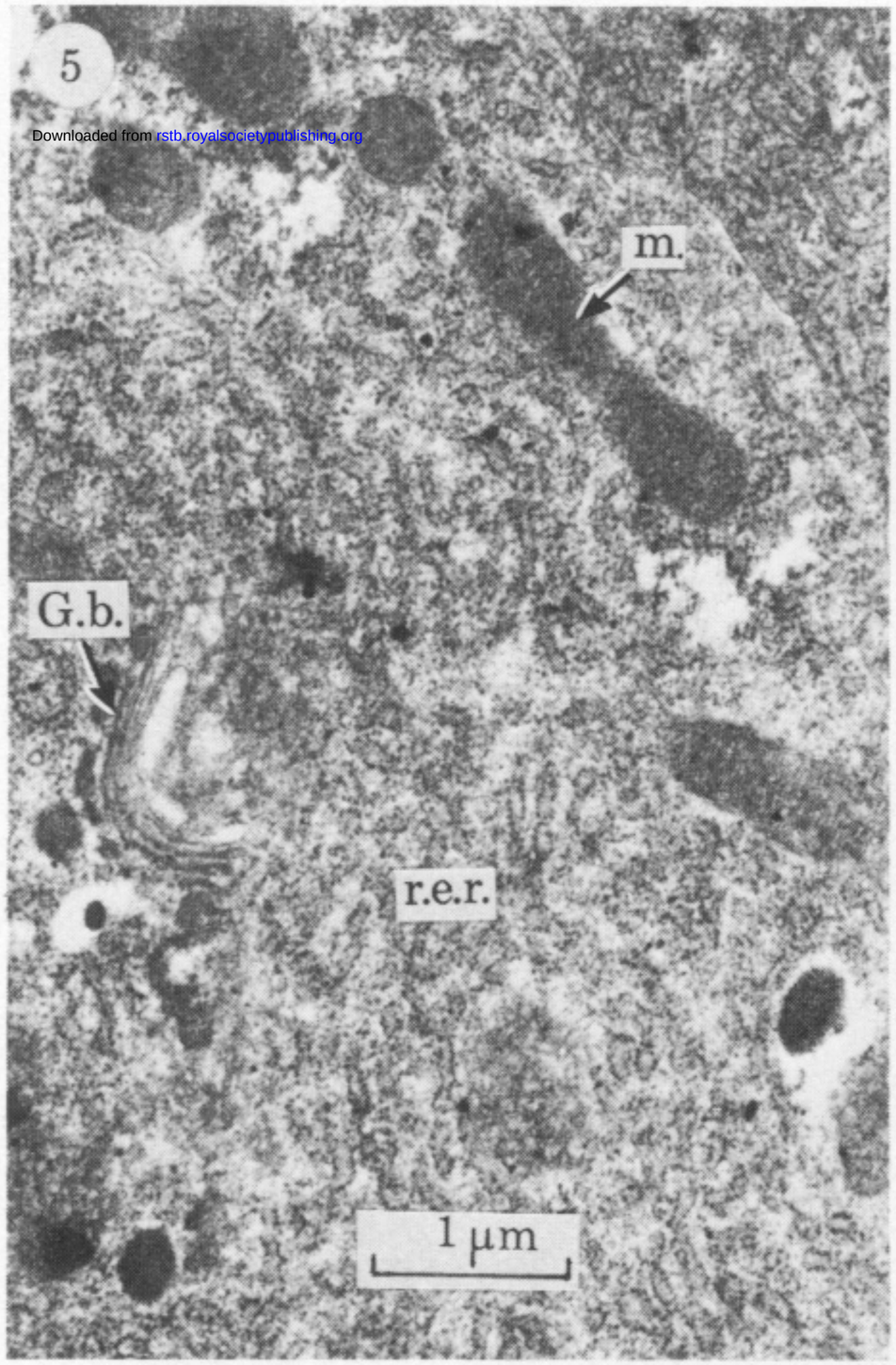


FIGURE 1. *Stegocephaloides christianiensis*, ventral caecum. Basal region of R/F cell with osmophilic lipid globules (l.g.) and dense rough endoplasmic reticulum (r.e.r.), showing discrete spiral muscle (s.m.) below the basement membrane (b.m.). Magn. ×7000.

FIGURE 2. *Stegocephaloides christianiensis*, ventral caecum. Epithelium consists of two cell types: R/F cells with long microvilli (l.m.), sub-spherical nuclei (n.) and osmophilic lipid globules (l.g.) basally, some of which have been lost in sectioning; tall B cells (forming epithelial crests) with stubby microvilli (st.m.), apical complex of membrane-bound vesicles (v.) and basal ovoid nucleus. Magn. ×1400.

FIGURE 3. *Stegocephaloides christianiensis*, ventral caecum. Apical regions of R/F cell showing Fe crystal (Fe c.) and long microvilli (l.m.), and B cell showing apical complex of membrane bound vesicles (v.). Magn. ×1800.

FIGURE 4. *Stegocephaloides christianiensis*, ventral caecum. R/F cell showing Fe crystal (Fe c.), apical calcium granules (Ca g.) and basal lipid globules (l.g.). Magn. ×3600.



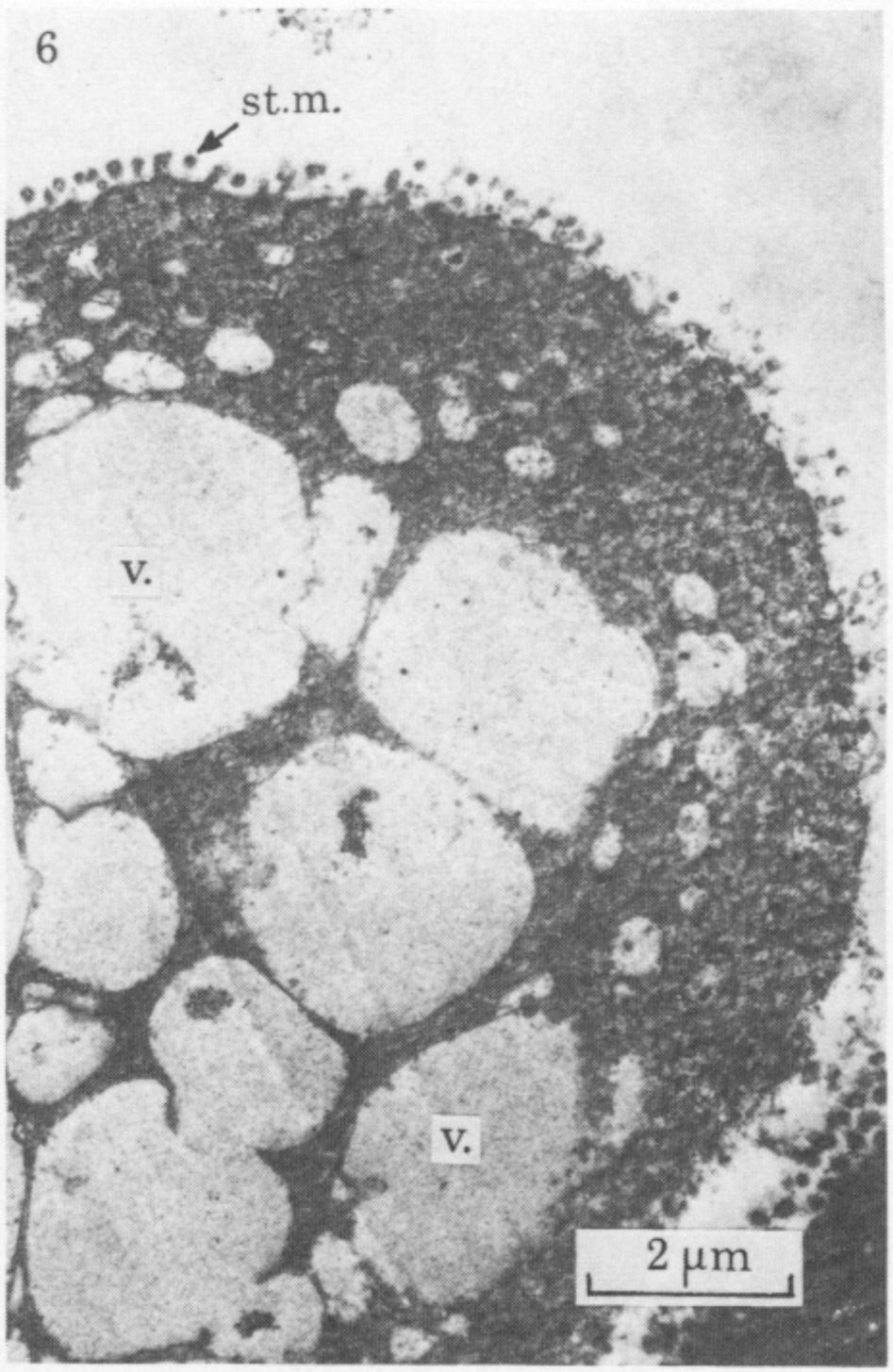
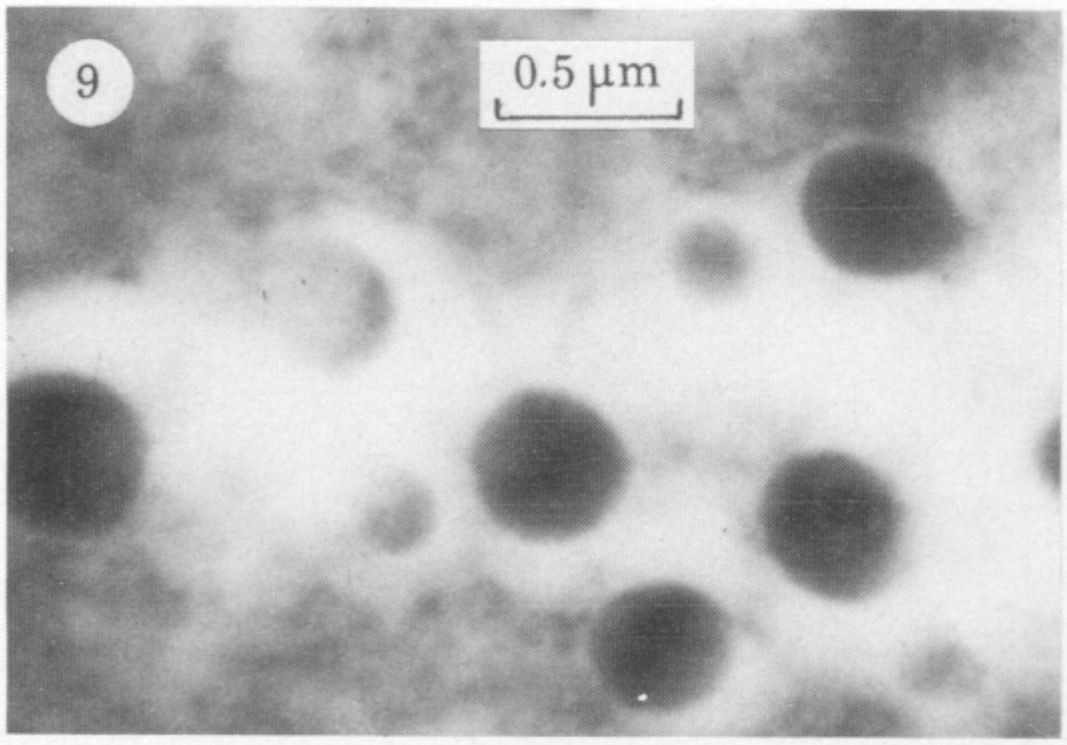
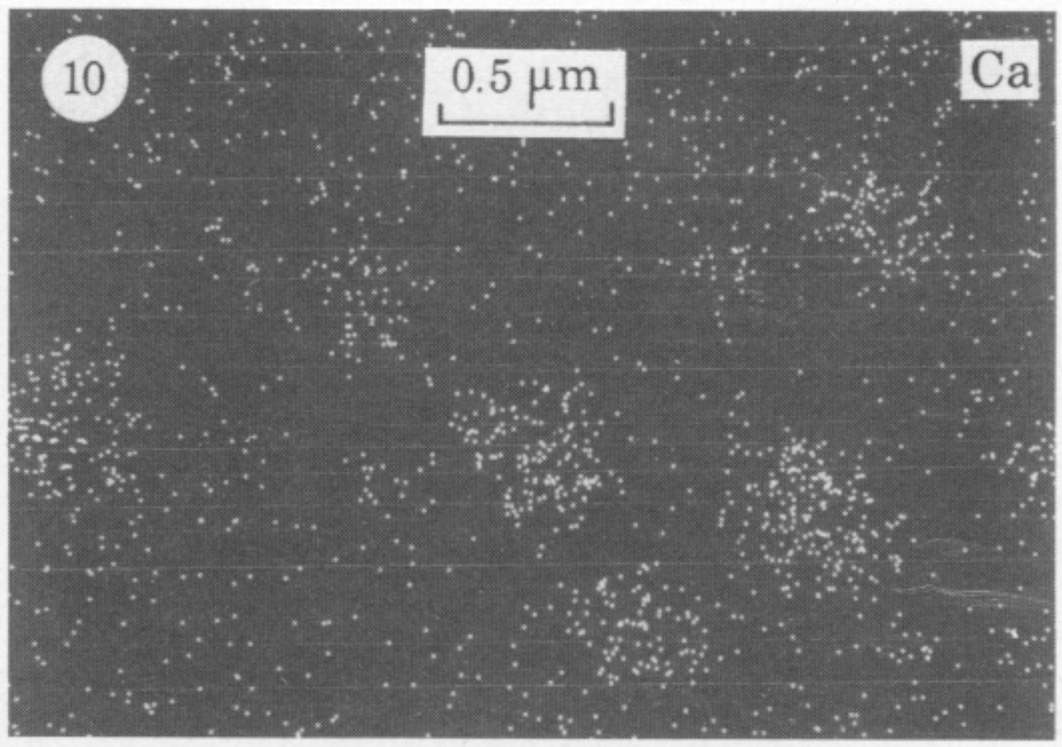
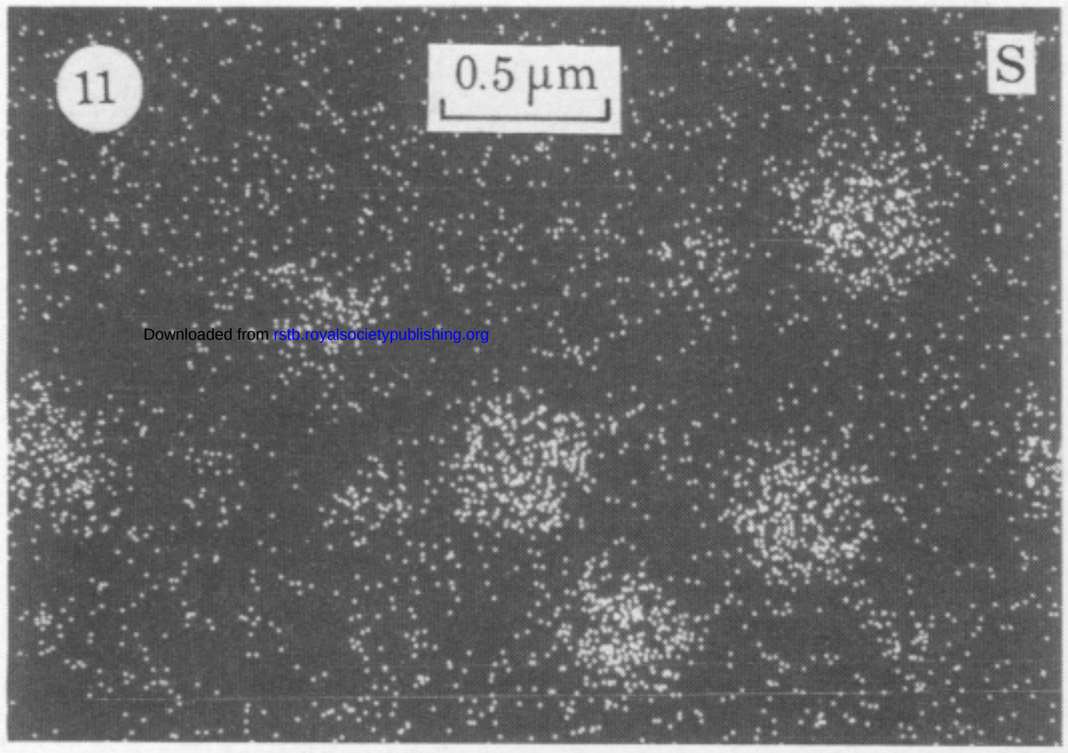


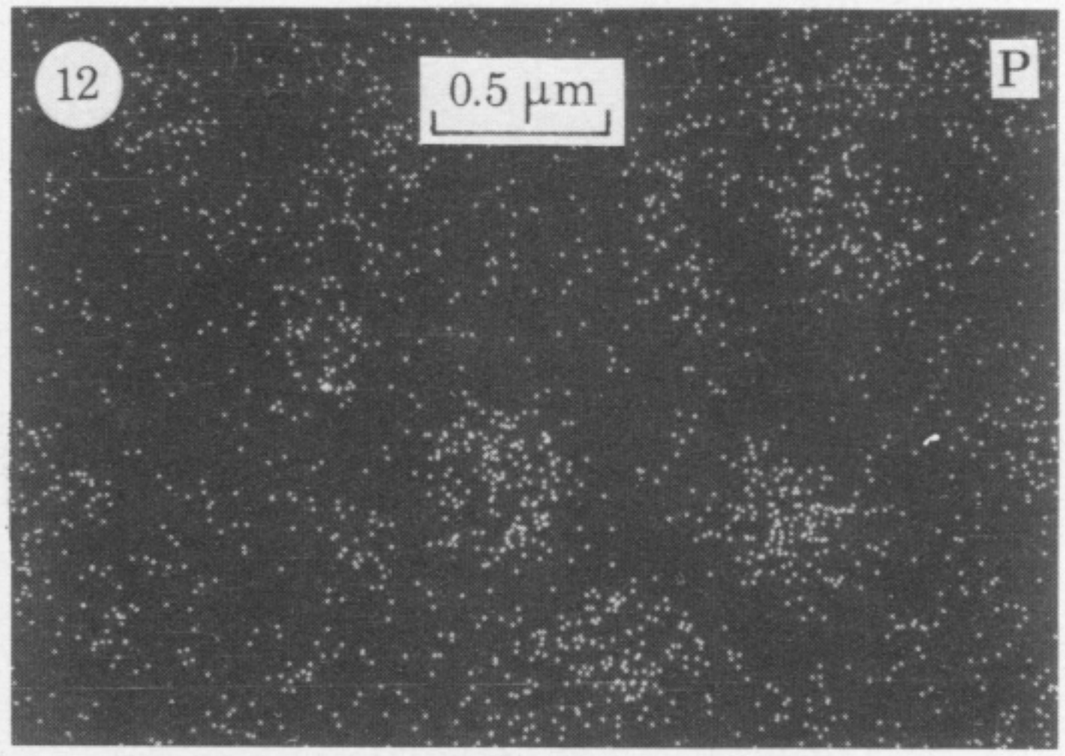
FIGURE 5. *Stegocephaloides christianiensis*, ventral caecum. R/F cell showing curved Golgi body (G.b.), dense rough endoplasmic reticulum (r.e.r.) and abundant mitochondria (m.). Magn. ×13000.

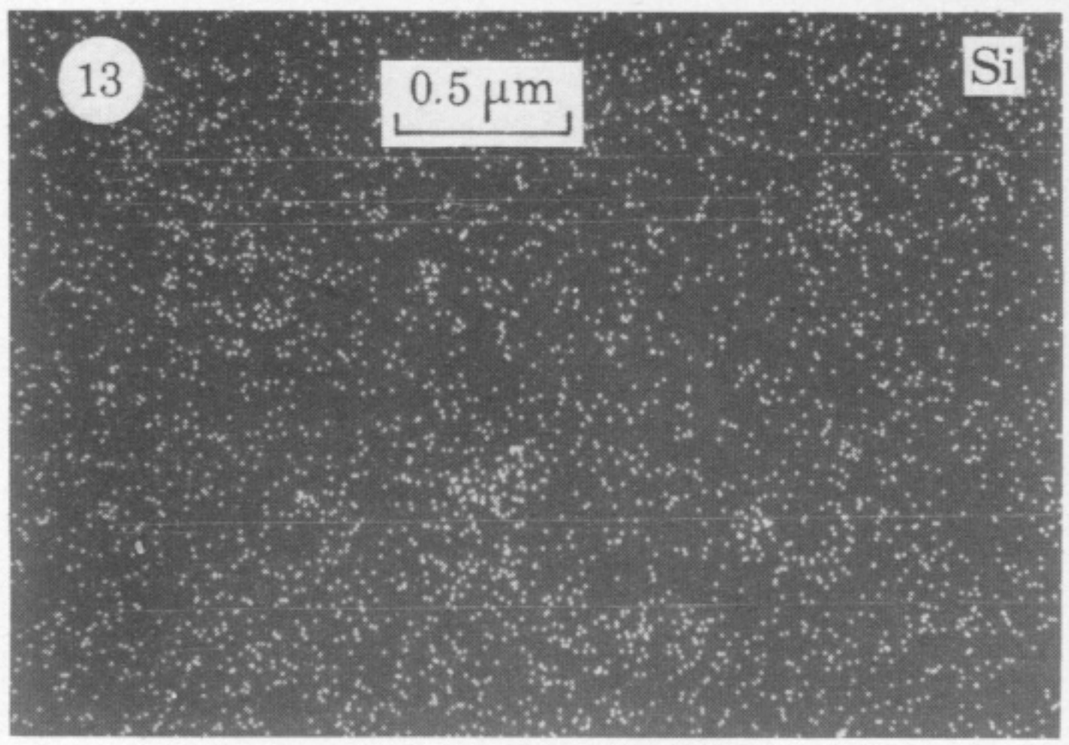
FIGURE 6. *Stegocephaloides christianiensis*, ventral caecum. Apical region B cell showing stubby microvilli (st.m.) and apical complex of coalescing membrane-bound vesicles (v.) with mostly electron-lucent contents. Magn. ×7000.











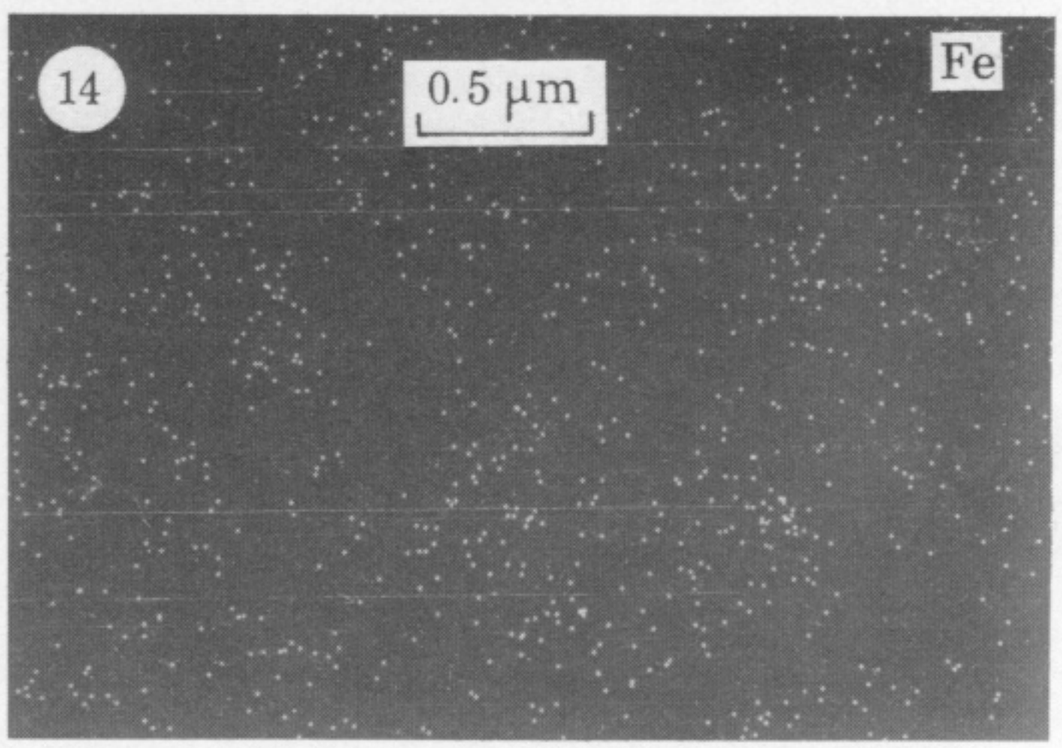
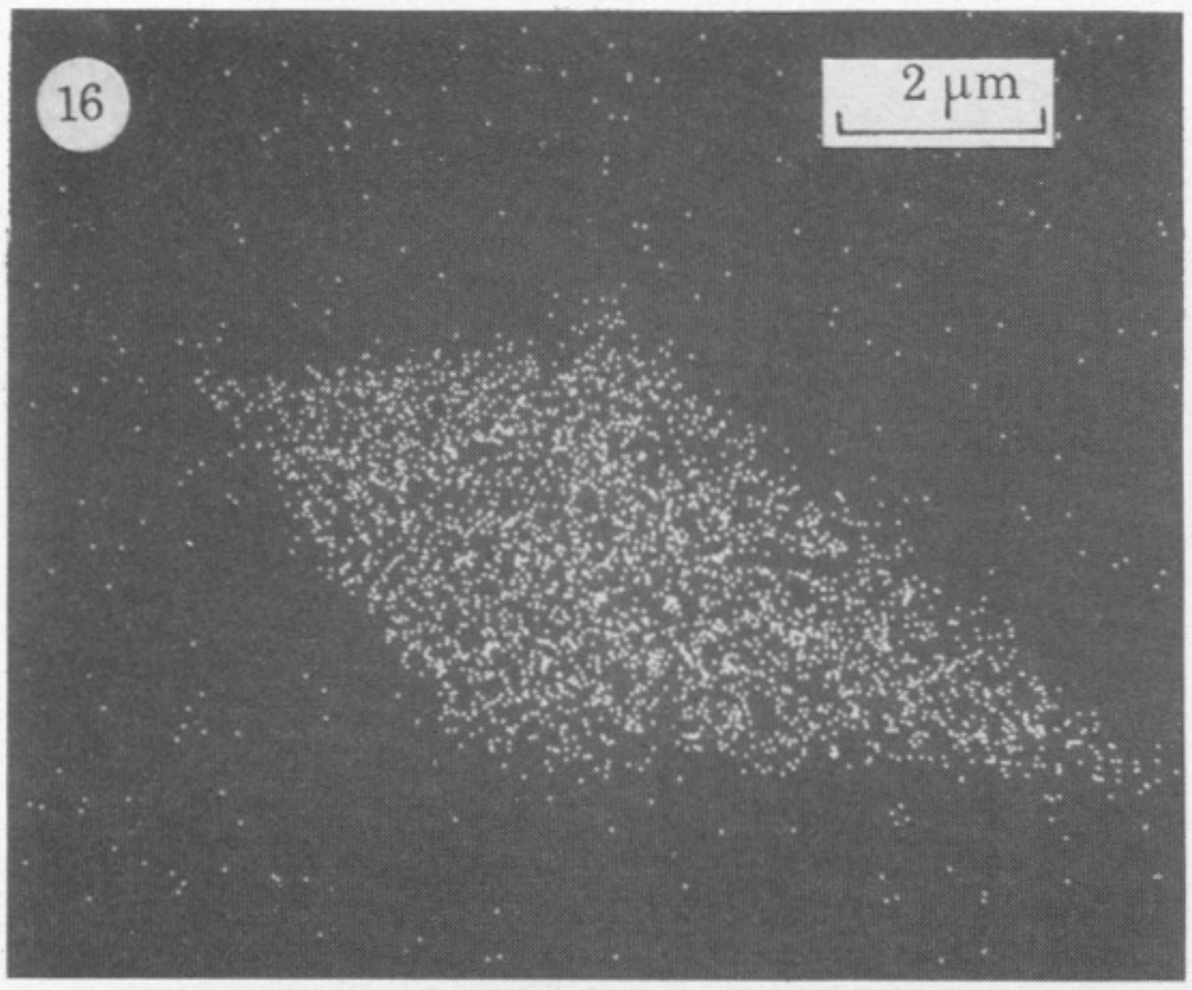
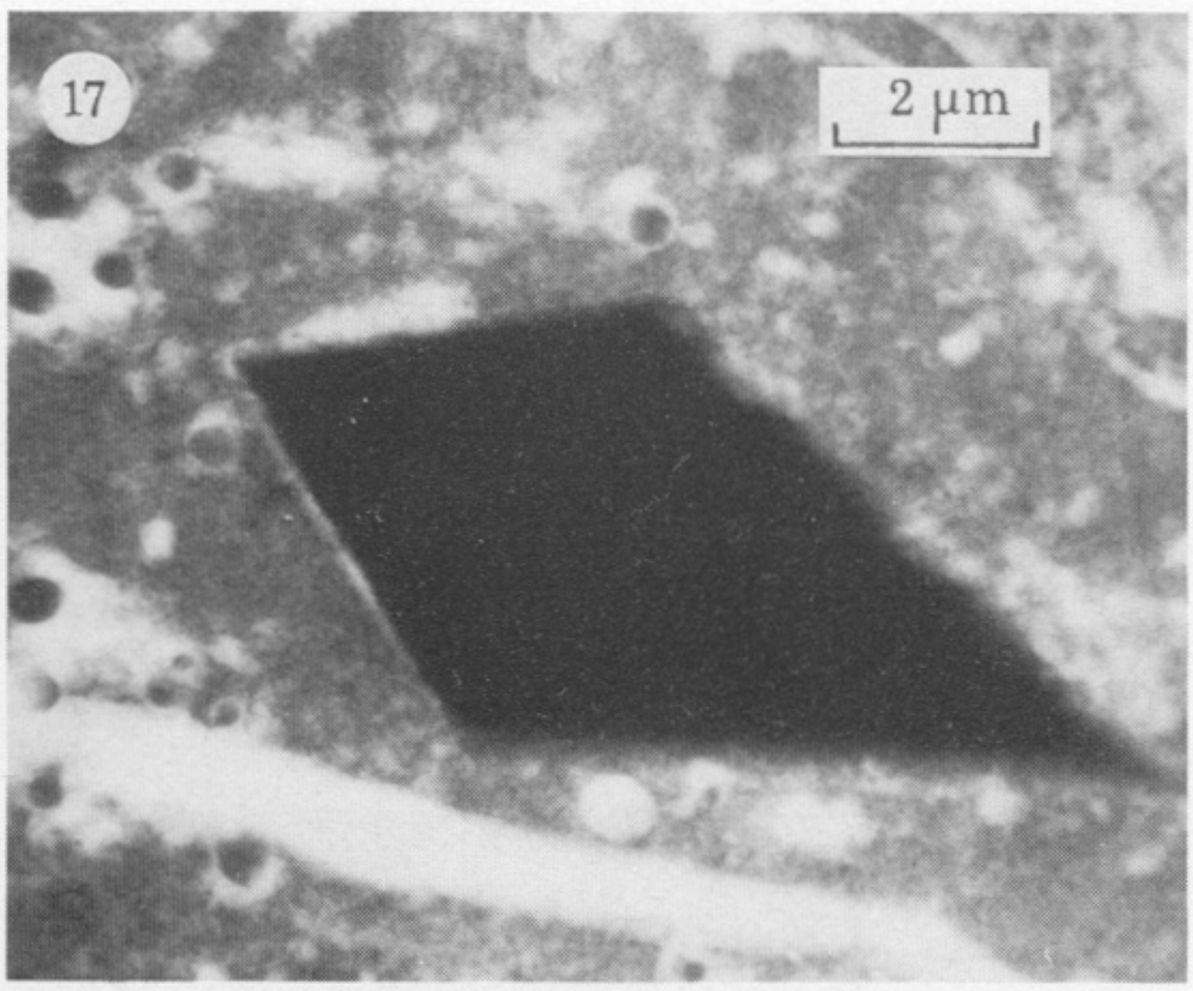
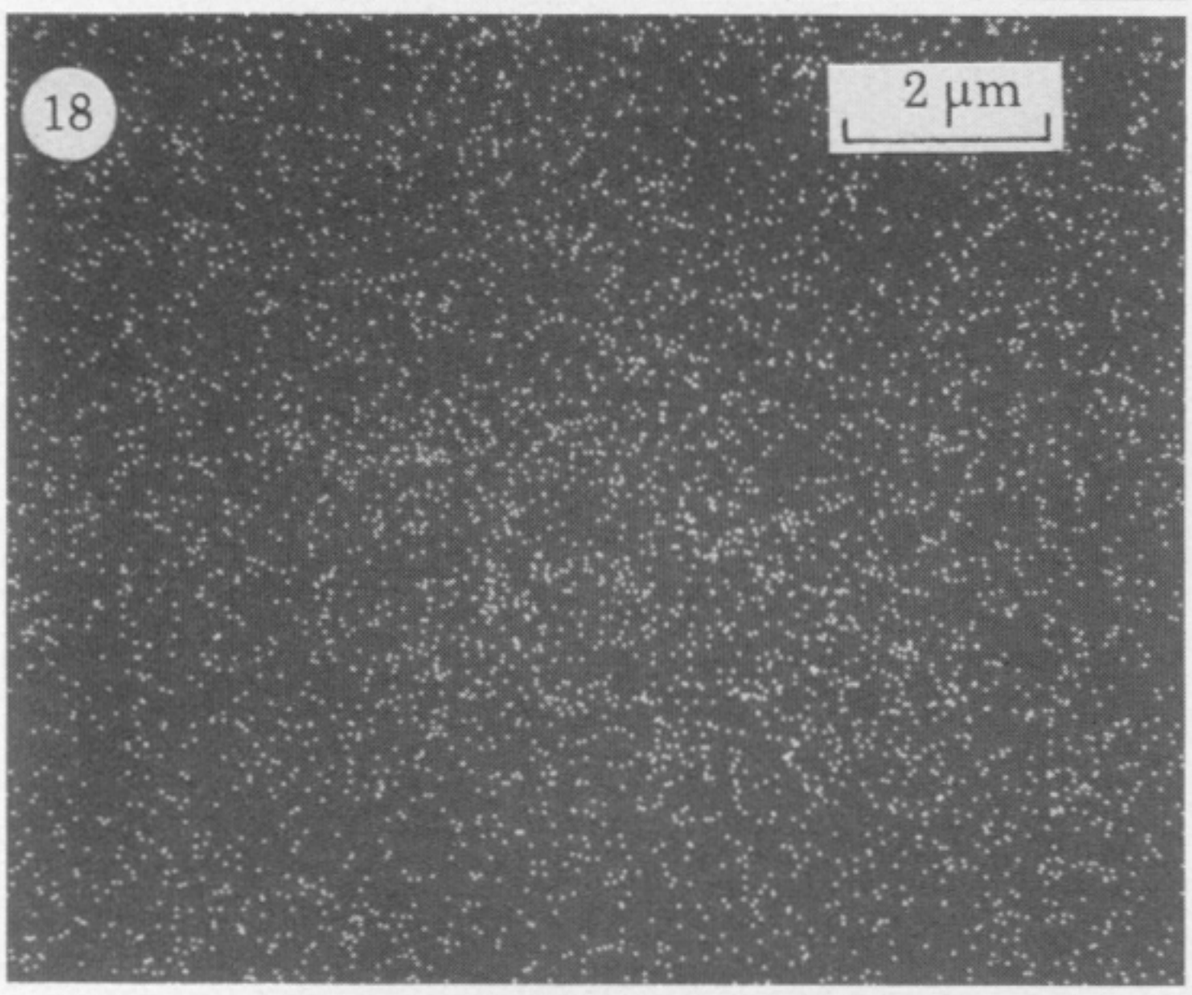


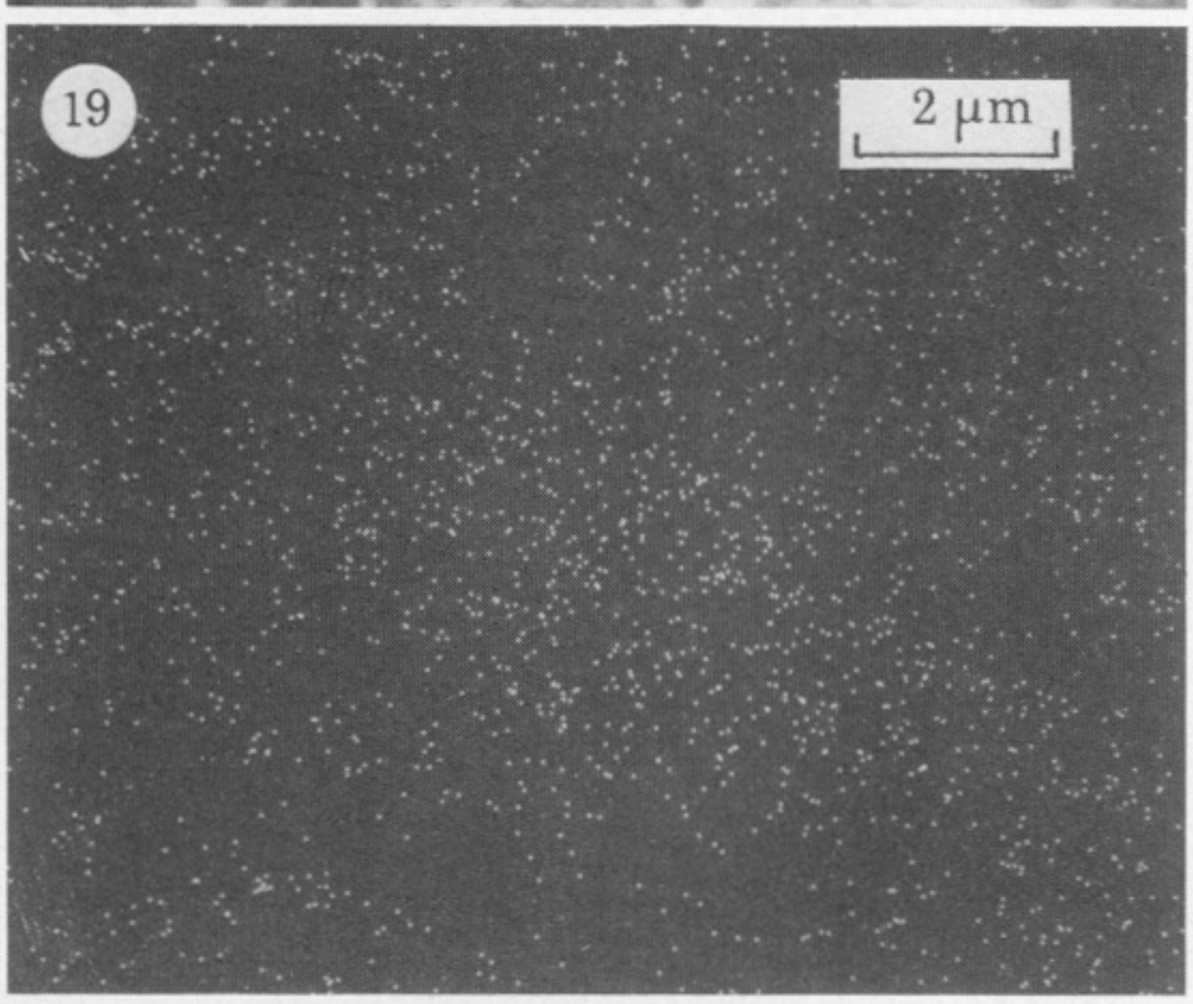
FIGURE 9. *Stegocephaloides christianiensis*, ventral caecum, s.t.e.m. micrograph of alcohol-fixed R/F cell calcium granules. Magn. ×22000.

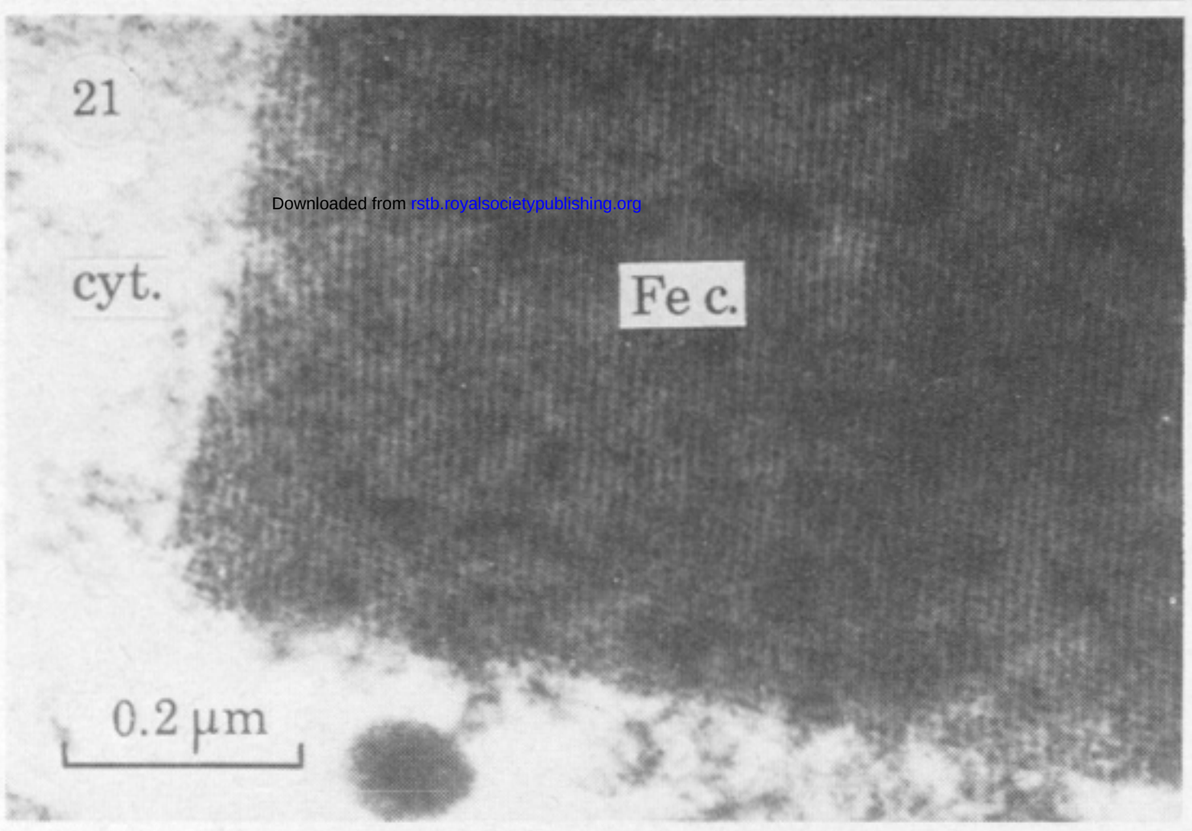
FIGURES 10–14. *Stegocephaloides christianiensis*, ventral caecum R/F cell calcium granules. X-ray distribution images for calcium (Ca) (figure 10), sulphur (S) (figure 11), phosphorus (P) (figure 12), silicon (Si) (figure 13) and iron (Fe) (figure 14) over the same area as figure 8 showing calcium, sulphur and (to a lesser extent) phosphorus to be localized within the granules but silicon and iron to be background. Magn. ×22000.

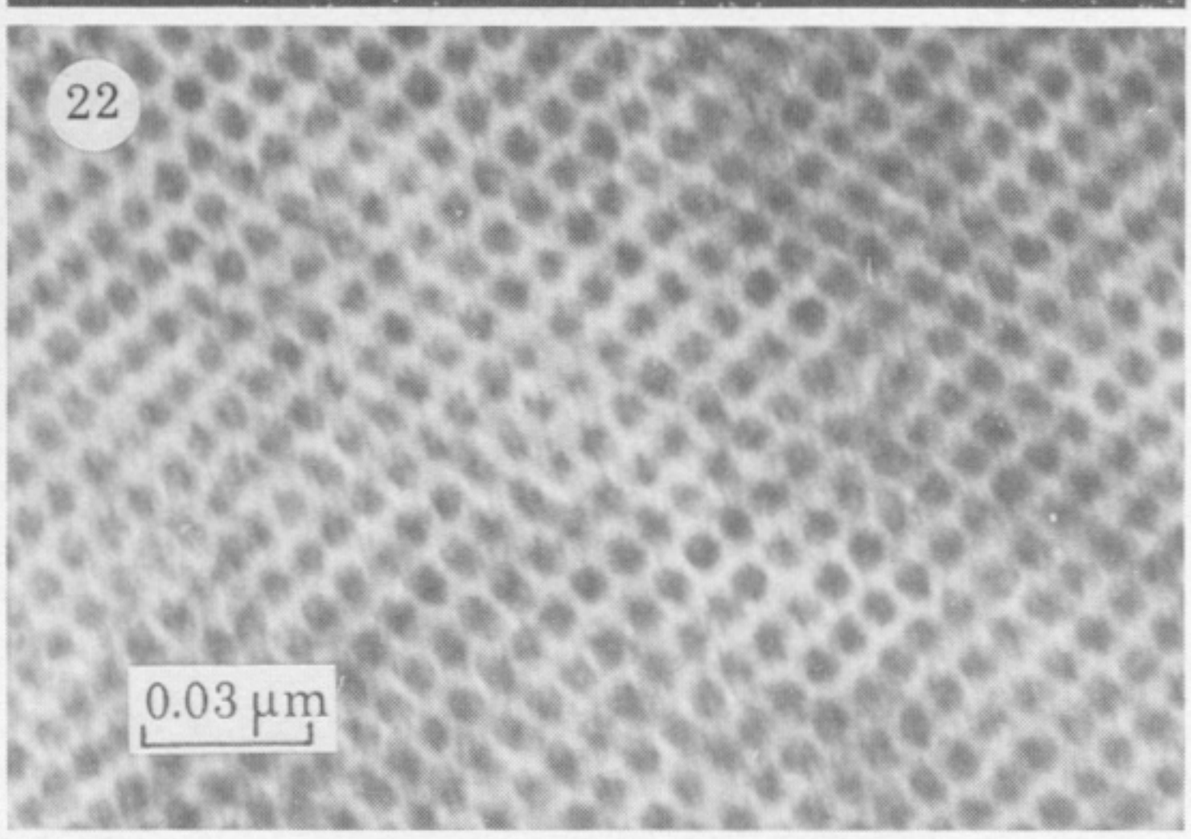


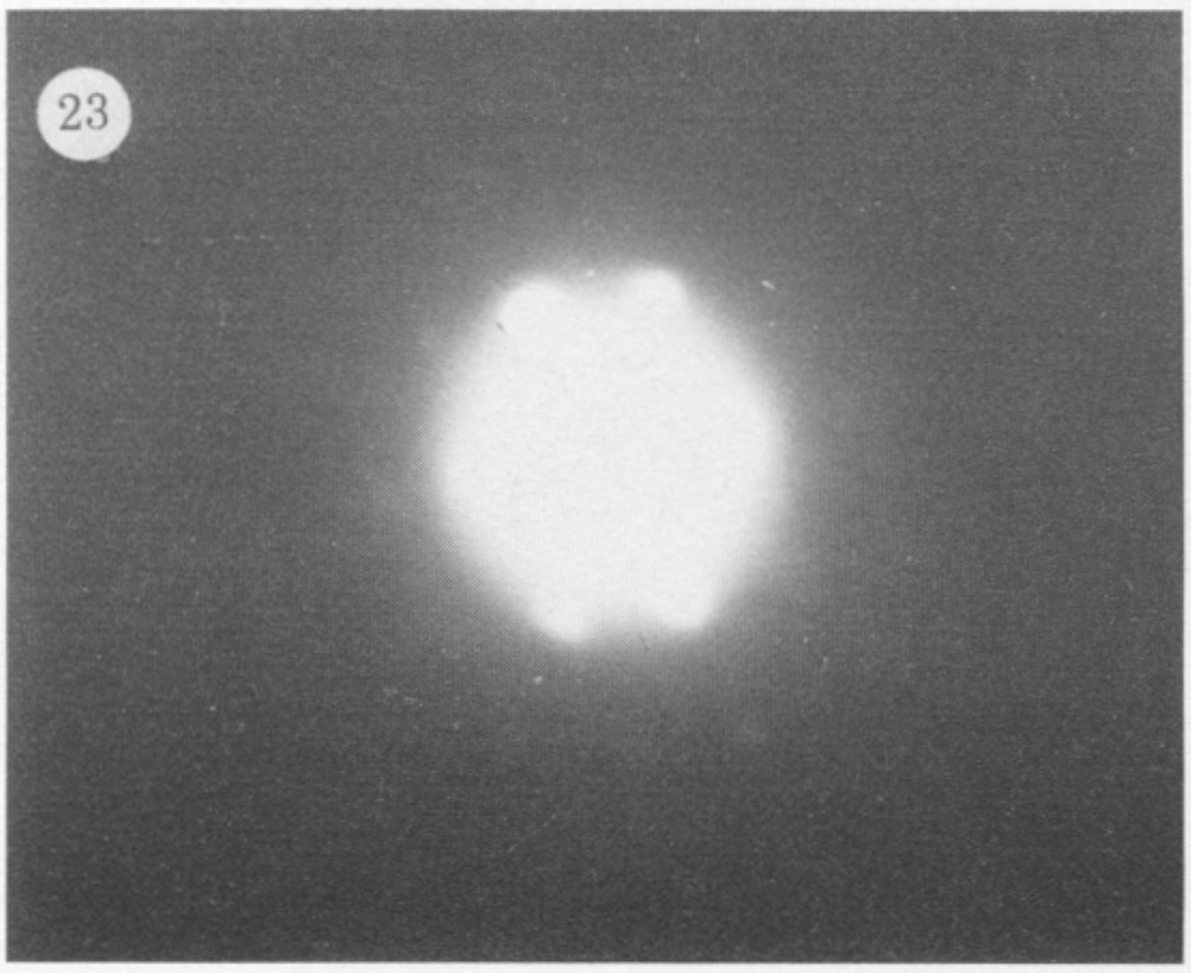


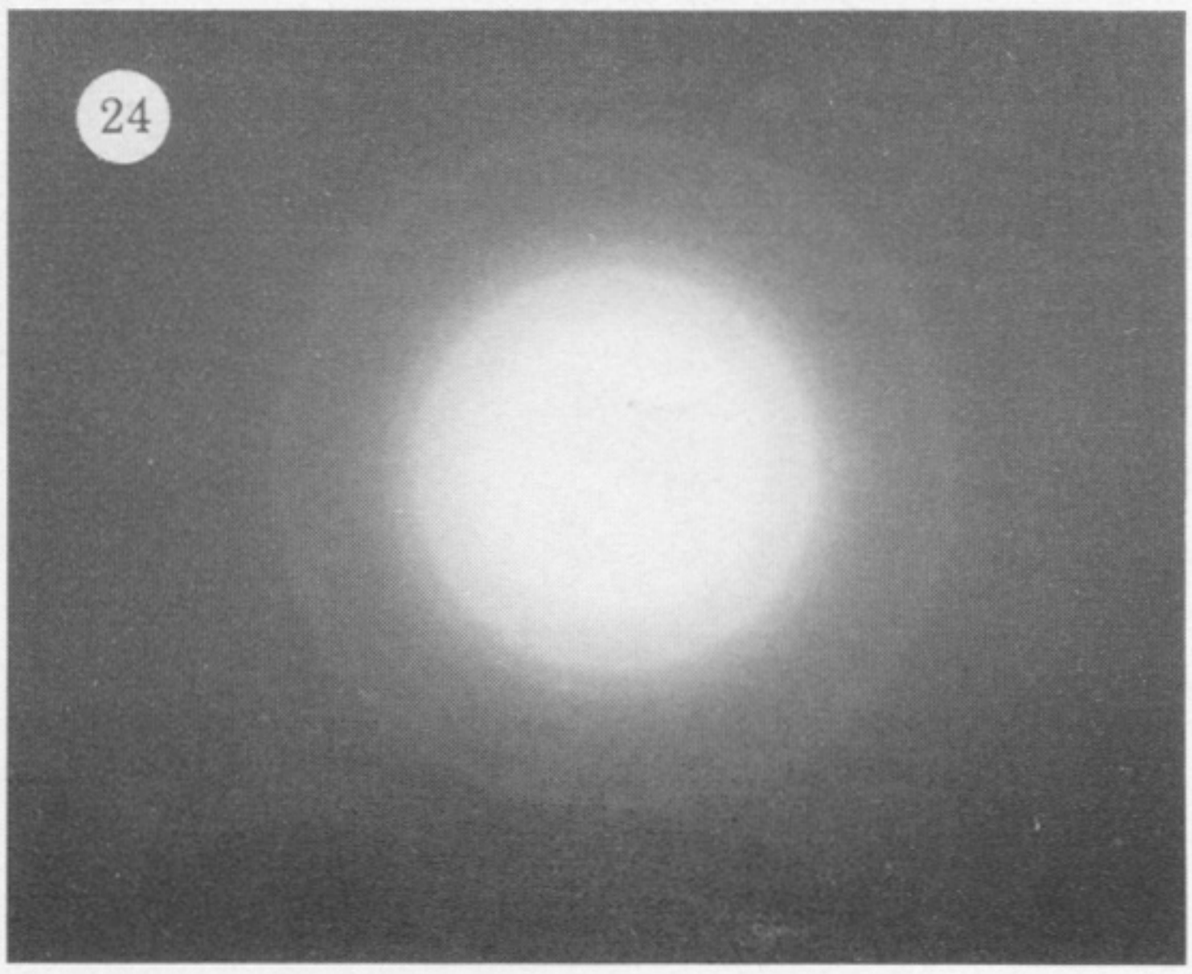






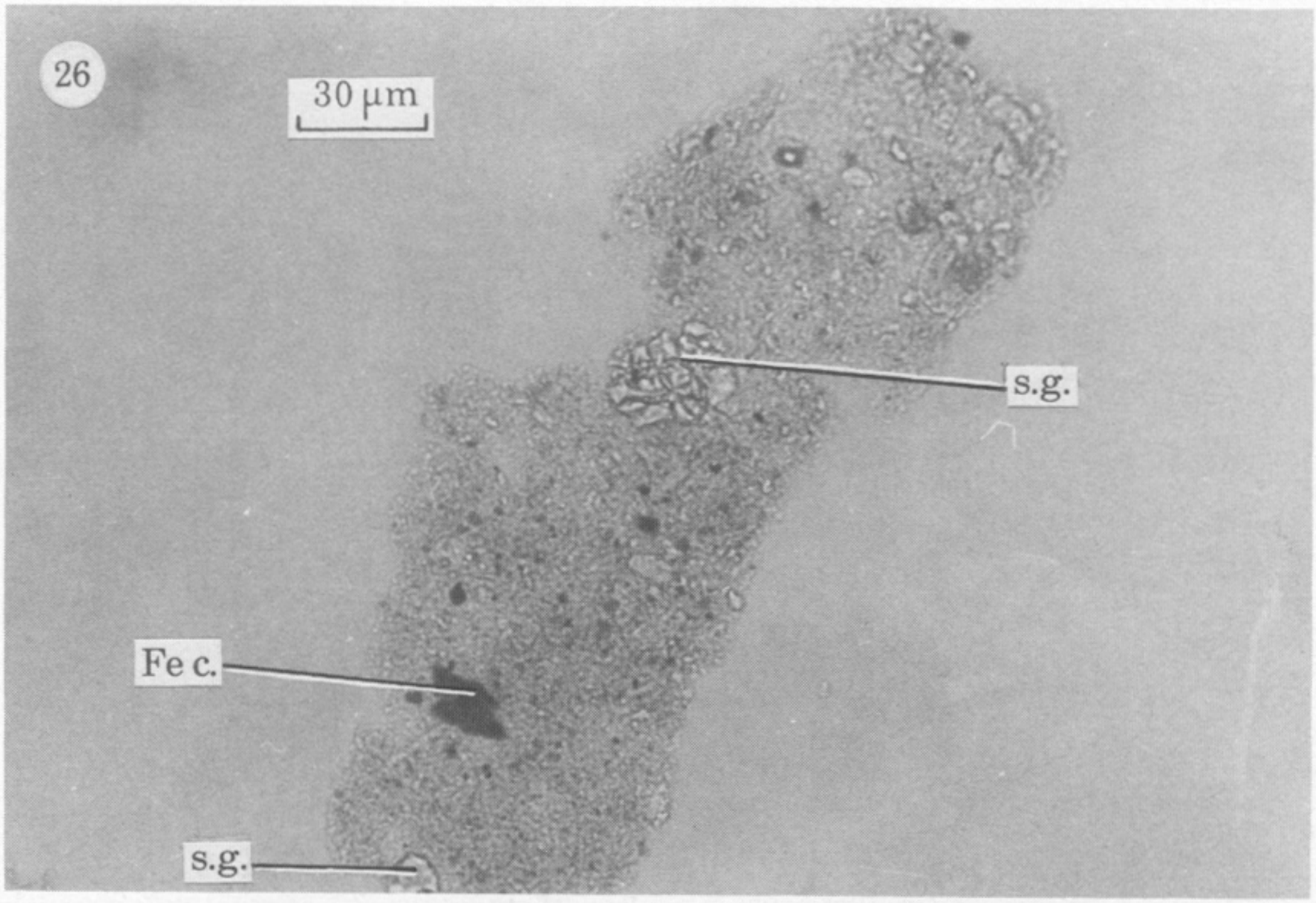


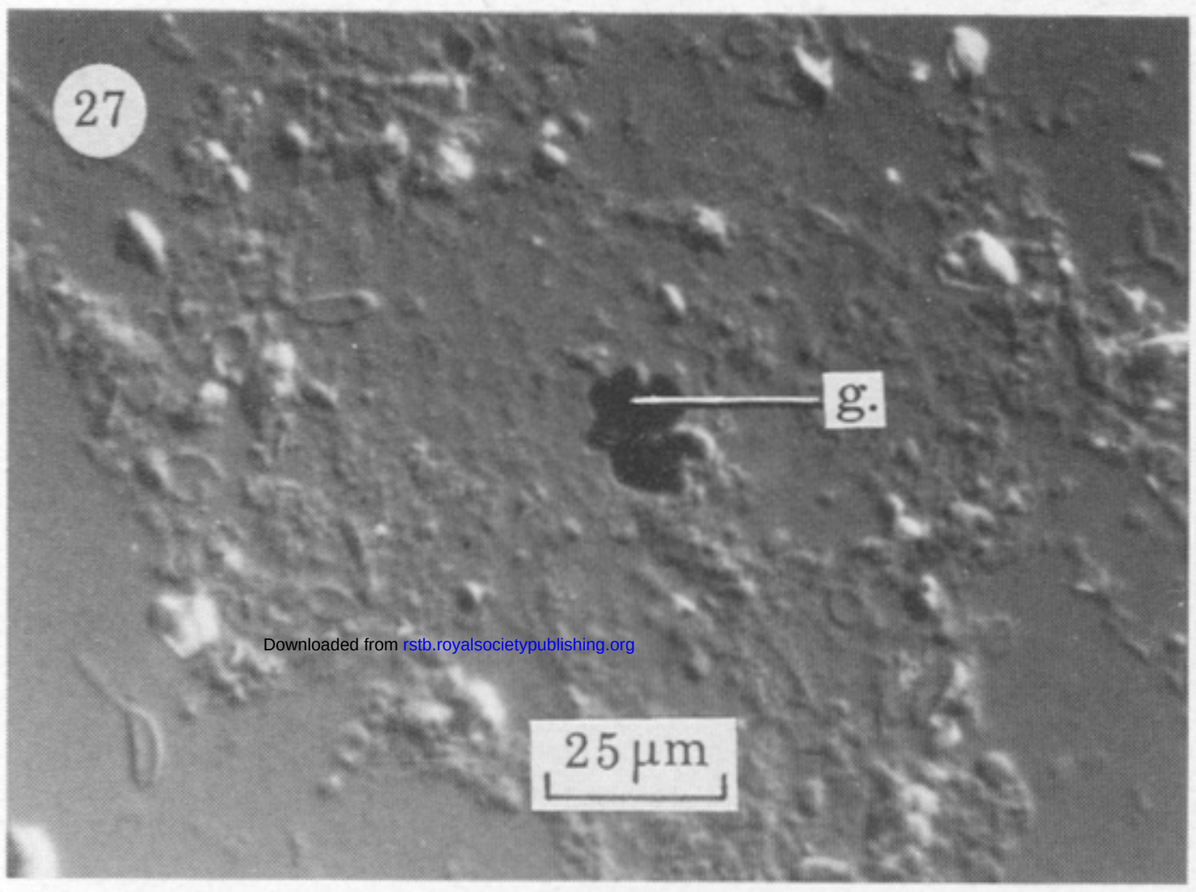


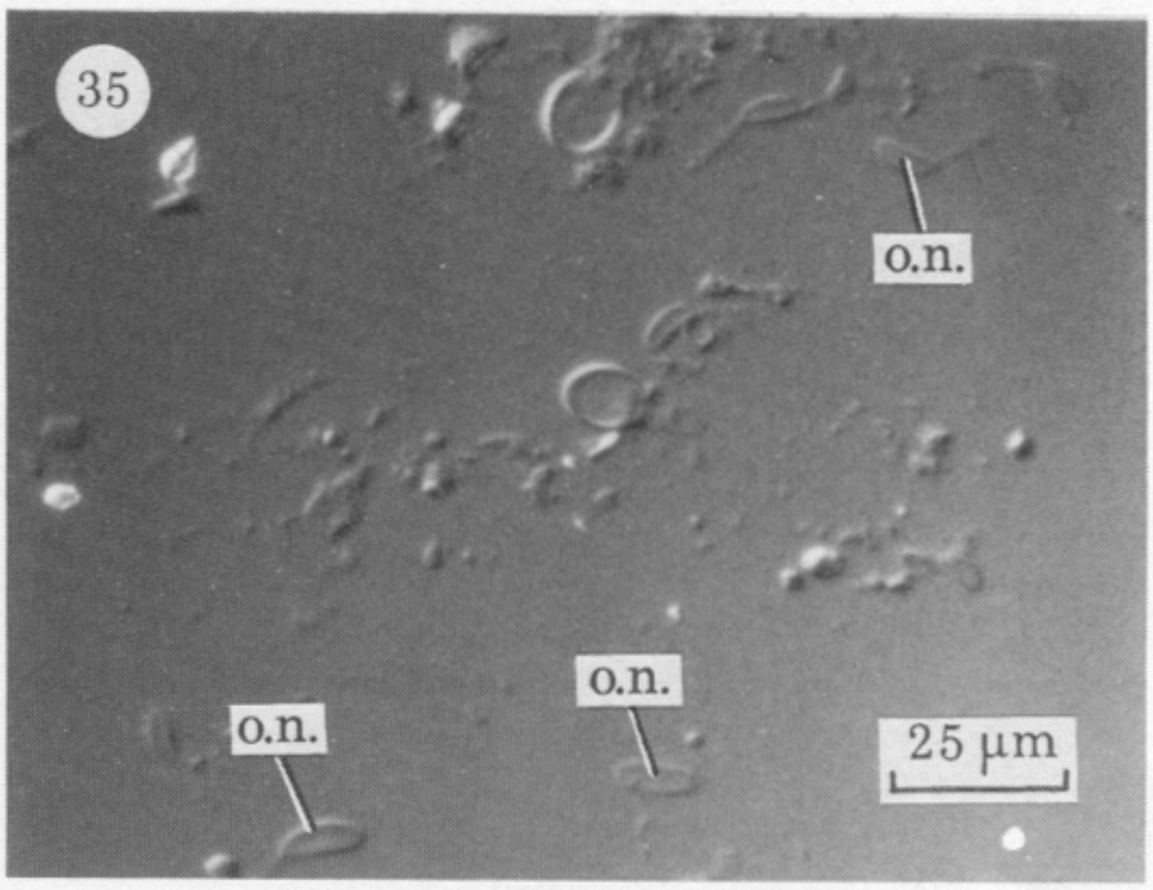


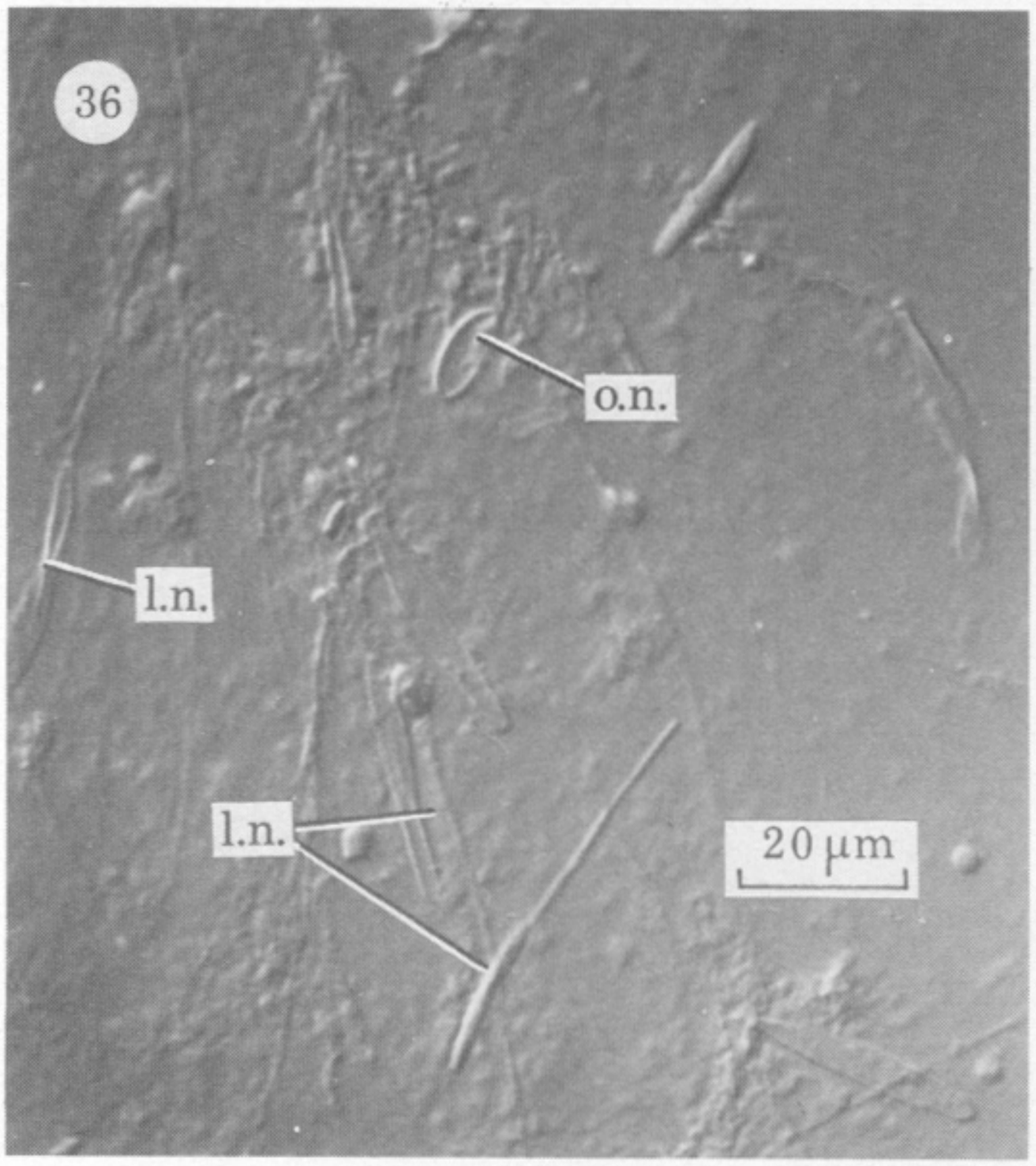
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

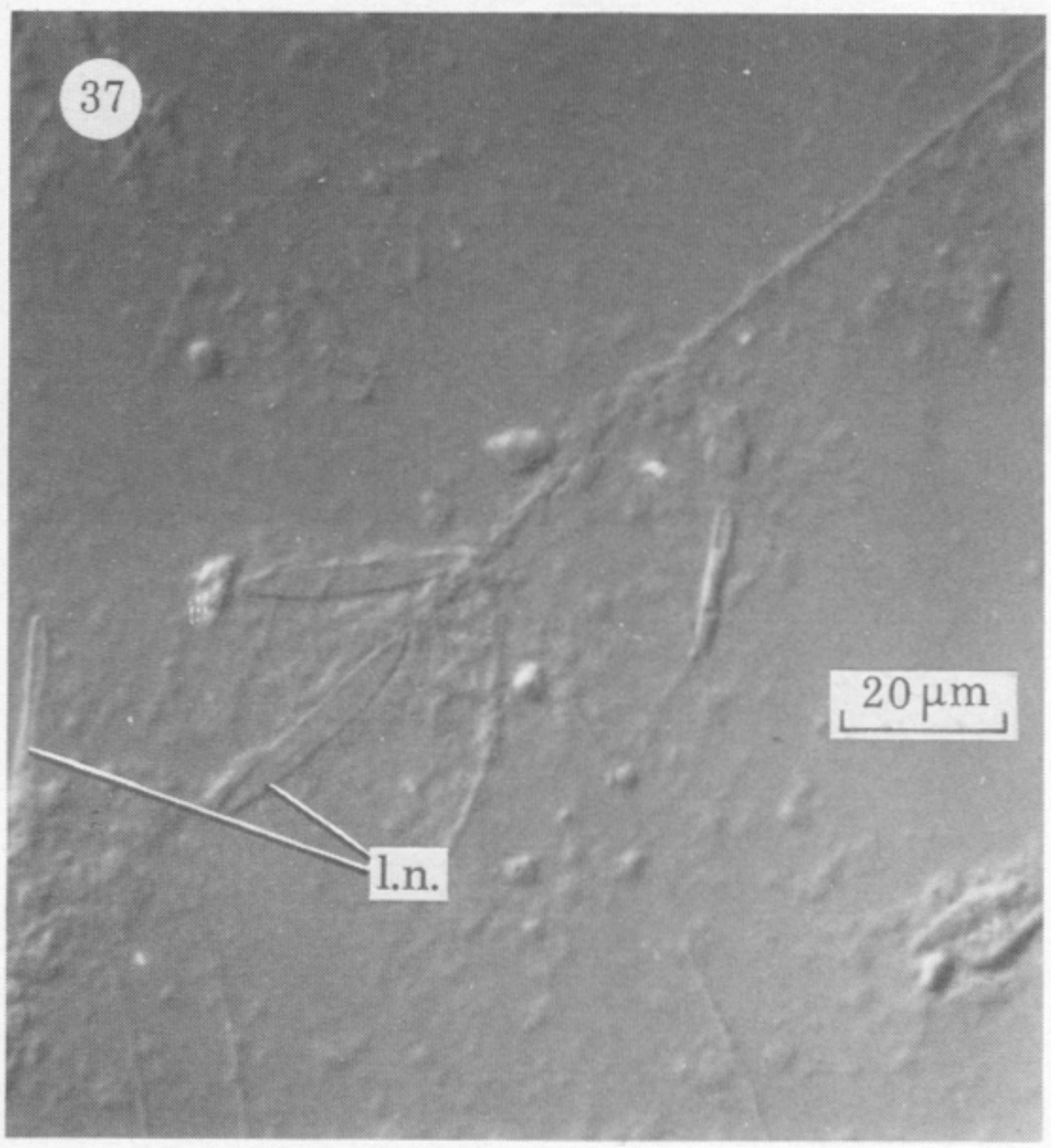
FIGURES 16–19, 21–24. For description see p. 227.





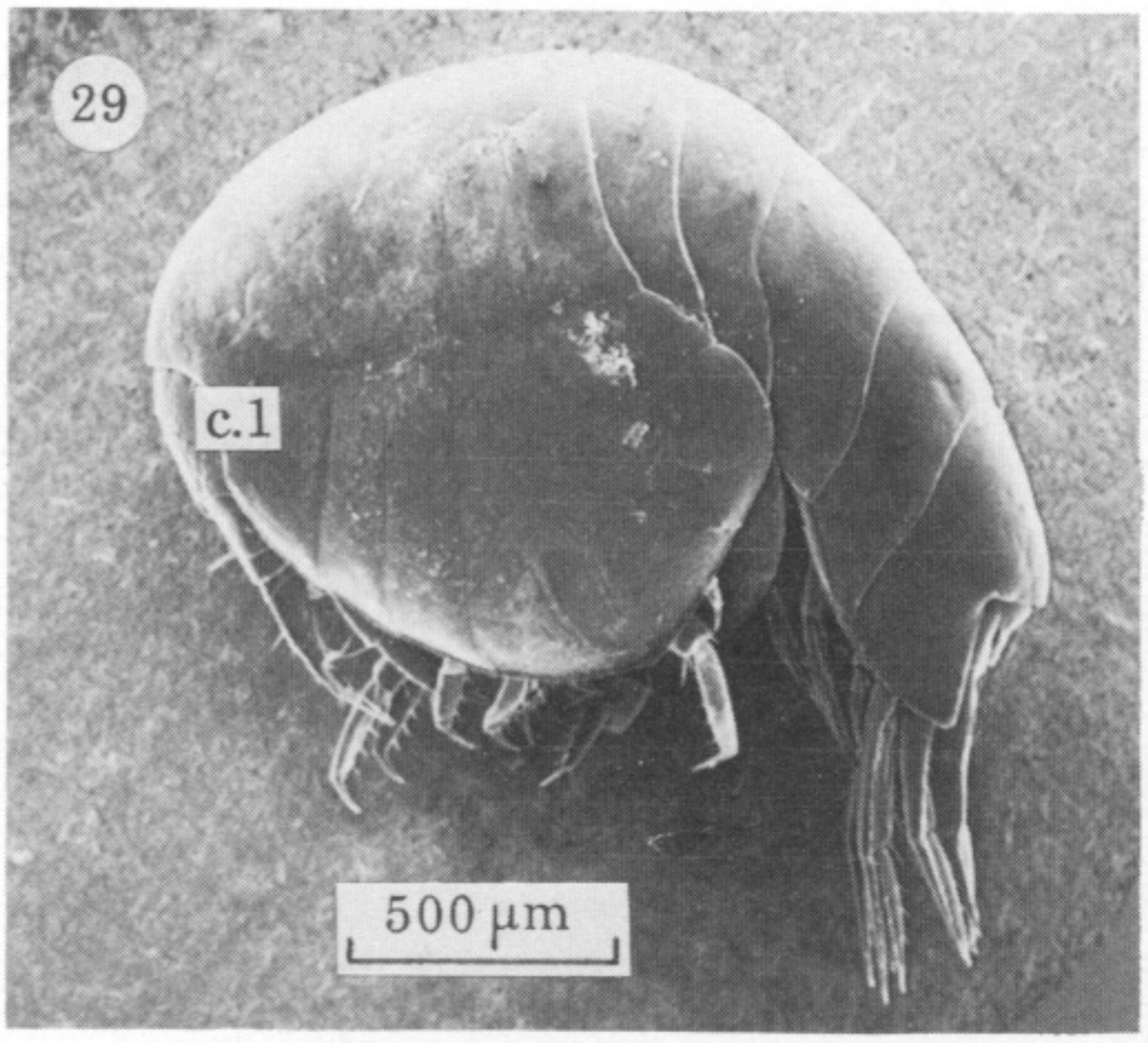


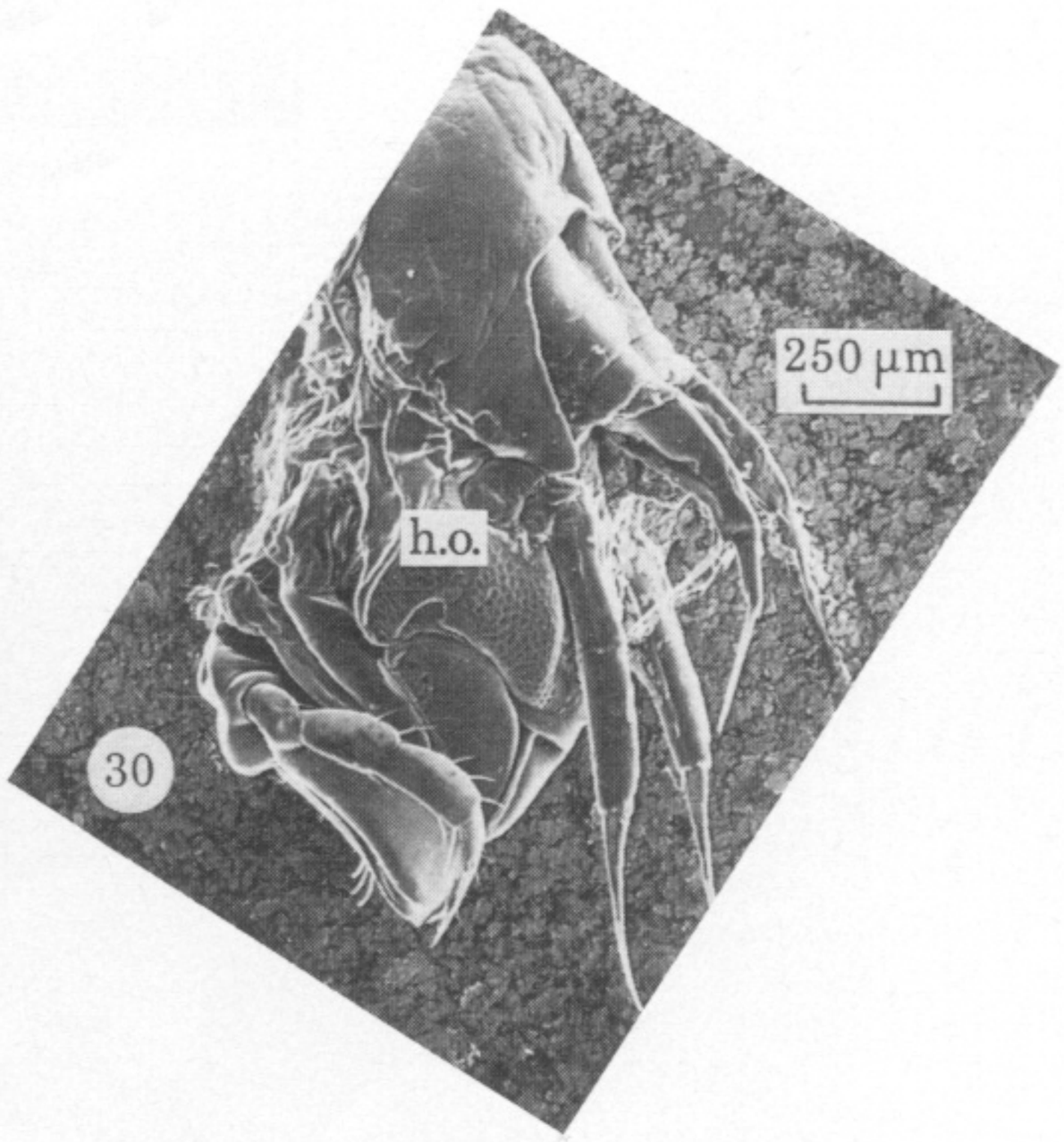


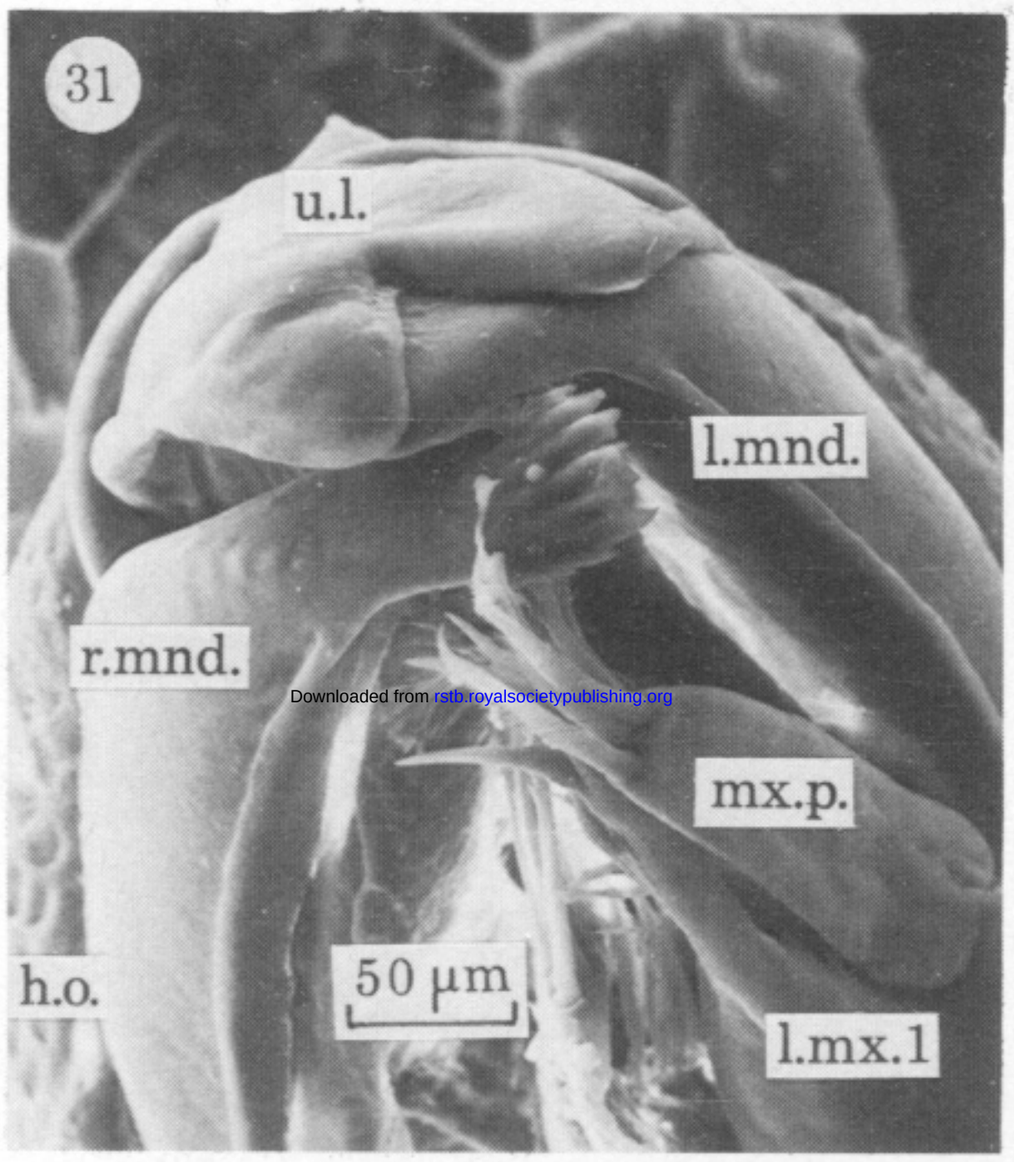


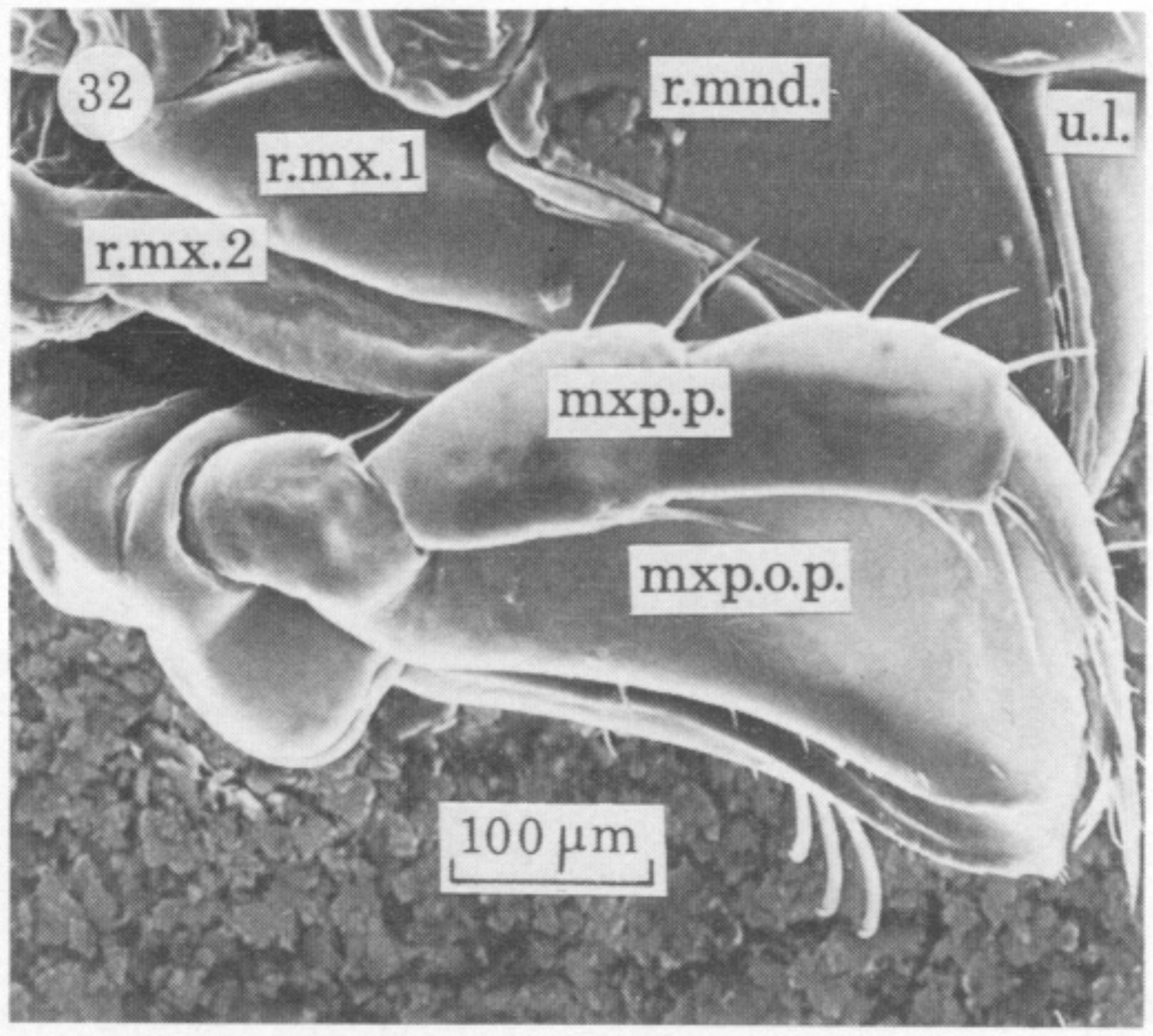
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

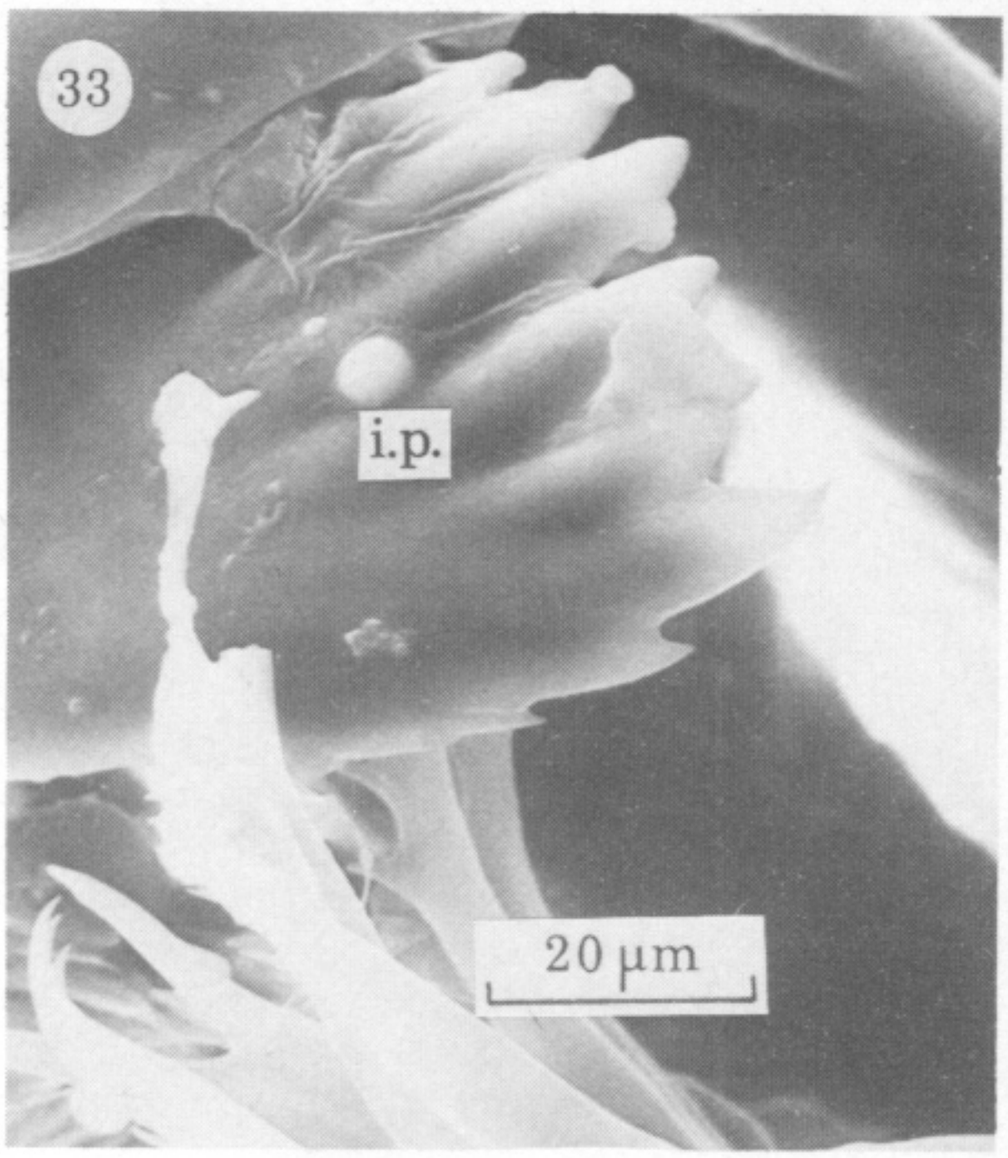
FIGURES 26, 27, 35–37. For description see p. 227.

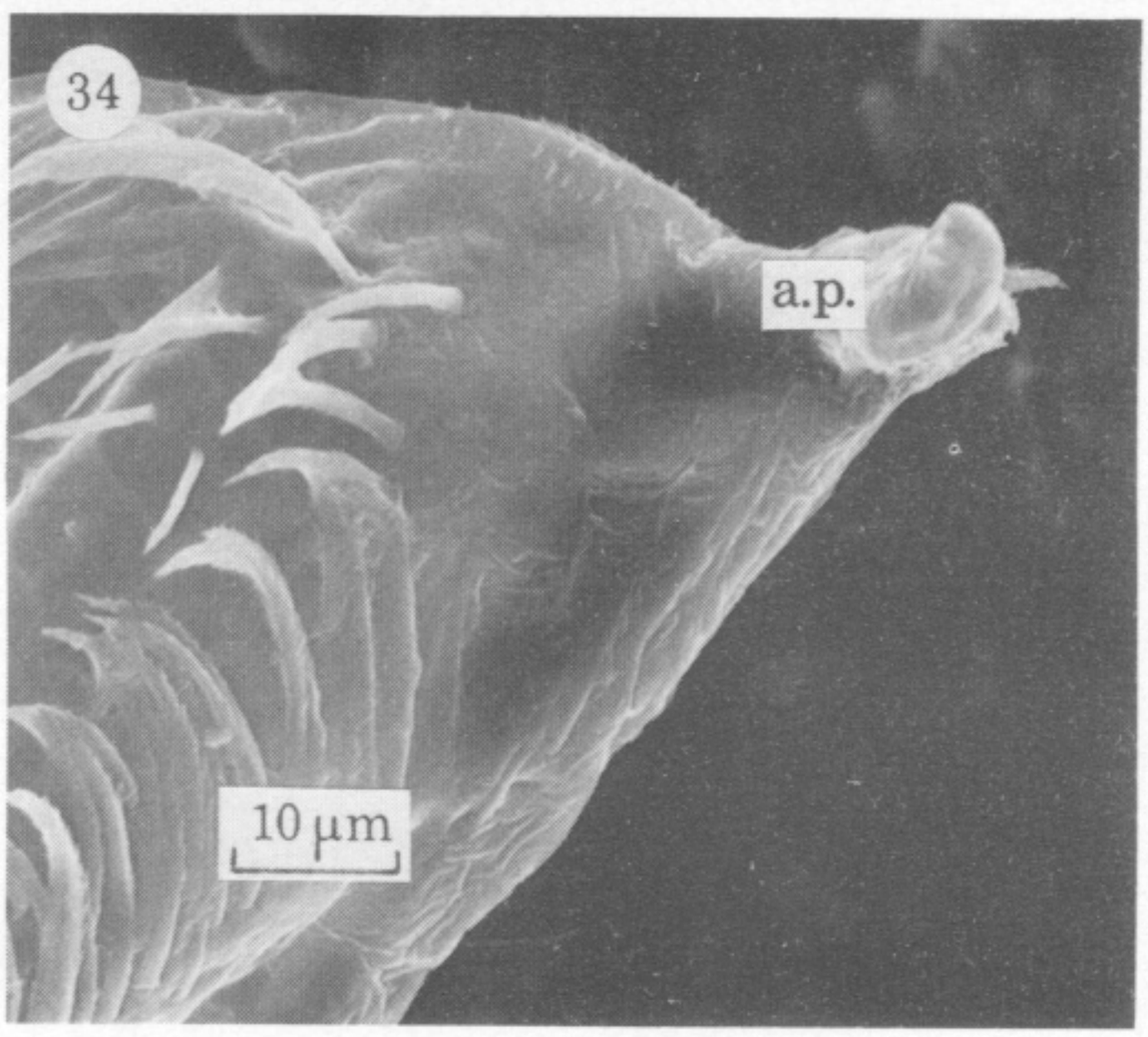












FIGURES 29–34. For description see opposite.