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Bioluminescent Australian Earthworms II.* Taxonomy and Preliminary Report of Bioluminescence in the Genera *Spenceriella, Fletcherodrilus* and *Pontodrilus* (Megascolecidae: Oligochaeta)

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Abstract

Bioluminescence is demonstrated in four species of *Spenceriella*, in *Fletcherodrilus fasciatus* and *F. unicus*, all being megascolecines from eastern Australia, and in the circummundane megascolecine *Pontodrilus bermudensis*, and is compared with that of the North American acanthodrile *Diplocardia longa*. The four *Spenceriella* species are placed in a new *cormieri* species-group in the subgenus *Spenceriella*. Of these, *S.*(*S.*) *cormieri*, *S.*(*S.*) *curtisi* and *S.*(*S.*) *noctiluca* are new species and *S.*(*S.*) *minor* (Spencer) is redescribed. The subgenus *Spenceriella* is redefined to include species lacking calciferous glands, and the subgenus *S.* (*Austroscolex*) is distinguished in lacking buccopharyngeal or other tufted nephridia anteriorly. *Fletcherodrilus* is redefined and a key to its four species provided. The synonymy and world distribution of *Pontodrilus bermudensis* Beddard is given. On electrical or tactile stimulation the seven described species, in *Spenceriella*, *Fletcherodrilus* and *Pontodrilus*, all exhibit spontaneous luminescence which is enhanced by addition of peroxide and which cross-reacts with *Diplocardia longa* luciferase and, usually luciferin. It is suggested that the luminescent system resides in the free chloragogen cells (elecocytes) in the coelomic fluid, in all except *P. bermudensis* in which luminescence is not cell-bound. Five species studied in the megascolecine genera *Heteroporodrilus*, *Spenceriella* (*Austroscolex*) and *Digaster*, and the glossoscolecid *Pontoscolex corethrurus*, are non-luminescent. Possible functions of luminescence are discussed.

Introduction

Extensive studies of the North American earthworm *Diplocardia longa* (Bellisario 1971; Bellisario *et al.* 1972; Ohtsuka *et al.* 1976; Rudie and Wampler 1978) and comparative studies between it and other species (Wampler, unpublished) prompted the authors to investigate luminescence in Australian earthworms and to compare the physiology and the biochemical systems of these worms with those of *D. longa*. In *D. longa* the bioluminescence is packaged in free chloragogen cells which are exuded in the coelomic fluid when the worms are excited. The reaction involves hydrogen peroxide and the simple aliphatic aldehyde, *N*-isovaleryl-3-amino propanal. It is catalyzed by *Diplocardia* luciferase (Rudie and Wampler 1978), a large protein containing copper. The components of this system have been purified to homogeneity (Rudie *et al.* 1976; Rudie 1977) and were available for these studies.

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Preliminary studies by Jamieson (unpublished) demonstrated the existence of brilliantly luminous Australian species from the Lamington Plateau in south-east Queensland and from Mt Warning in northern New South Wales. In addition, a weakly luminous species *Digaster keasti* from Fraser I. has been described (Jamieson 1977*a*). In this latter paper a list was given of all the oligochaetes for which luminescence has been reported.

In January and February 1978, the authors collaborated, as part of a United States–Australian Cooperative Science Program, in the investigation of light production in earthworms from south-east Queensland. Luminescence was demonstrated in four species assignable to *Spenceriella*, from the Lamington Plateau and adjacent Mt Tamborine, in the widespread large species *Fletcherodrilus fasciatus* and *F. unicus* from various localities, and in the strandline euryhaline circummundane species *Pontodrilus bermudensis* from Peel I. in Moreton Bay. In each case the luminescent system from these worms had several features in common with that of *D. longa* from North America. No luminescence was found in *Heteroporodrilus lamingtonensis*, unidentified *Heteroporodrilus* species, in an undescribed species of *Spenceriella* (*Austroscolex*) from the Bunya Mountains, in *Digaster binnaburra* and *D. bradburyi bunyaensis* (Megascolecidae) or in the circummundane *Pontoscolex corethrurus* (Glossoscolecidae).

With the exception of the well known *Pontodrilus bermudensis*, light-producing species were either new to science or were in need of redescription. This account therefore commences with a taxonomic description of the luminescent species, and notes on their luminescence. A summary of the general features of their bioluminescence follows the taxonomy. The depth of taxonomic treatment at supraspecific level will be varied as appropriate.

Materials and Methods

Diplocardia luciferin and luciferase were prepared as previously described (Ohtsuka *et al.* 1976; Rudie 1977). Hydrogen peroxide (3%) was reagent grade and was diluted with buffer to give 0·03% solutions. All buffer salts and other reagents were of the best grade available.

The luminescence of the live specimen and the *in vitro* luminescence reactions were measured and monitored with a photometer designed and constructed by the Bioluminescence Laboratory (G. J. Faini, unpublished). The unit was routinely calibrated with a radioluminescence secondary standard (Hastings and Weber 1963) which was itself calibrated relative to the luminol chemiluminescence reaction (Lee *et al.* 1966).

Suspensions of coelomic fluid, extracts and assays were prepared with a buffer of 0·1 M potassium phosphate (pH 7·5 at 4°C) containing 0·125 g litre^{-1} sodium azide and 75 mg litre^{-1} dithiothreitol. The buffer is hypertonic relative to coelomic cells from *D. longa* (Bellisario 1971) and was, therefore, used to prepare washed cell fractions for this work. After a worm had been stimulated with a hand-held magnetogenerator and its luminescence observed, 2 ml of this buffer were used to suspend and dissolve the coelomic fluid. Extracts of washed particulate material or whole coelomic fluid were prepared with a Ten Broek glass homogenizer. All extracts were kept at ice temperature during testing and were stored at −20°C.

In vitro tests for cross-reactions and stimulation were carried out by taking 0·1 ml of extract in a small glass test tube and placing it in the photometer. After the basal level of luminescence had been observed, volumes of test reagents were injected into the test tube while it was still in the photometer. A continuous chart record was maintained. The following volumes and concentrations of test reagents were used: 0·1 ml distilled water; 0·05 ml of 0·3% hydrogen peroxide; 0·05 ml *Diplocardia* luciferase (~0·1 units ml^{-1}); 0·05 ml of 0·25 mg ml^{-1} *Diplocardia* luciferin. All reagents were prepared by use of the extraction buffer.

Descriptive Format and Abbreviations

The attributes employed and their sequence in the generic and specific descriptions conform with those in previous works (e.g. Jamieson 1971*a*).

Abbreviations in the illustrations are: *c*, cerebral ganglia; *ca.g*, calciferous gland; *d.v*, dorsal blood vessel; *dv.c*, dorsoventral commisural vessel; ♀, female pore; *g.m*, accessory genital marking; *int*, intestine; *lo.h*, latero-oesophagael heart; ♂, male pore; ♂*f*, sperm funnel; *n.c*, ventral nerve cord; *np*, nephropore; *o*, ovary; *oe*, oesophagus; *o.f*, oviducal funnel; *peri*, peristomium; *pr.d*, prostate duct; *pr.g*, glandular portion of prostate; *pro*, prostomium; *sp.amp*, spermathecal ampulla; *sp.d*, spermathecal duct; *sp.div*, spermathecal diverticulum; *sp.p*, spermathecal pore; *s.v*, seminal vesicle; *v.d*, vas deferens; *ves*, nephridial bladder; *ves. div*, diverticulum of nephridial bladder.

Abbreviations for institutions in which specimens are lodged are: AM, Australian Museum, Sydney; BMNH, British Museum (Natural History), London; BJ, Jamieson Collection; HM, Zoologisches Museum und Staatsinstitut, Hamburg; QM, Queensland Museum.

Systematics

Tribe **MEGASCOLECINI** Jamieson

Genus **Spenceriella** Michaelsen

Spenceriella Michaelsen, 1907. Emend. Jamieson, 1977*b*.

Diagnosis (see also Jamieson 1977*b*)

A pair of combined male and prostatic pores in segment XVIII. Gizzard in V. Caudally with nephrostomes in the same segments as their nephridial bodies in addition to a median preseptal funnel in each segment. No setae median to the male pores.

Remarks

Spenceriella is currently under revision and will receive large numbers of species hitherto attributed to the heterogeneous congeries *Megascolex* (Jamieson, unpublished). The new species added in the present account lessen the facility with which the genus may be divided into its two subgenera *Spenceriella* and *Austroscolex*. The subgenus *Spenceriella* as defined by Jamieson (1977*b*) was readily distinguished, by possession of three or four pairs of extramural calciferous glands, together with anterior enteronephric (bucco-pharyngeal) tufted nephridia, from *Austroscolex*, which lacks these. However, the four species described here have bucco-pharyngeal nephridia but lack calciferous glands. On these grounds they could be placed in either subgenus but the evidence is that their closest affinities are with the subgenus *Spenceriella*, in which they are here included as a *cormieri* group of species. The evidence for this is the constancy of absence of bucco-pharyngeal nephridia in *Austroscolex*, as originally defined, the generally lesser development of the gizzard in the latter subgenus, the affinities between two *Megascolex* species which it is beyond the scope of this work to discuss in depth but which must be briefly mentioned because of their relevance to the status of the two subgenera. The first is *Megascolex tenax* (Fletcher, 1886) which has three pairs of subspherical calciferous glands, in XI–XIII, and buccal tufted nephridia (personal examination of Old Newington material, Australian Museum reg. No. AM WI364). This is clearly referable to the subgenus *Spenceriella*. The second is *Megascolex wiburdi* Boardman, 1943, in which the oesophagus, though swollen in X–XIII, bears no diverticula but has (enteronephric?) anterior tufts. The

genital field and general anatomy of *M. wiburdi* leave little doubt of its close affinity with *M. tenax* (and with *M. jenolanensis* Boardman, 1943, which is morphologically close to *M. tenax*). Thus the presence of bucco-pharyngeal nephridia appears superior to presence or absence of calciferous glands as an indicator of relationship, and for distinguishing *Spenceriella* from *Austroscolex*.

For taxonomic convenience it will nevertheless be useful to recognize the homogeneous *notabilis* group, for species in which bucco-pharyngeal enteronephry is accompanied by presence of calciferous glands, and a (heterogeneous) *cormieri* group, for species which lack calciferous glands, while recognizing that some members of the *cormieri* group (notably *wiburdi*) may have their closest affinities within the *notabilis* group. Such species-groups do not have formal taxonomic status but have served a useful purpose in previous publications.

Subgenus **Spenceriella** Michaelsen, emend.

Diagnosis

Gizzard usually large, sometimes reduced. Bucco-pharyngeal meronephridial tufts present in anterior segments. 3 or 4 pairs of dorsolateral extramural calciferous glands typically present (*notabilis* species-group) or absent (*cormieri* species-group).

Distribution

Victoria, New South Wales, South Australia, Tasmania, Queensland: Torresian Province, Kosciuskan Provinces (Queensland Plains Province, Eastern Montane–Coastal Province, Southern Province), Tasmanian Province; Western Subregion, Darling Basin Province. (Terminology based on Kikkawa and Pearse 1969.)

Type-species: *Diporochaeta notabilis* Spencer, 1900.

In the following checklists asterisked species are bioluminescent. The other species have not been tested for luminescence.

Spenceriella (*S.*) *notabilis* species-group

Dorsolateral calciferous glands present.

For species see Jamieson (1974). Victoria, South Australia, New South Wales, Tasmania.

Also *S.* (*S.*) *jenolanensis* (Boardman, 1943), comb. nov. New South Wales: Cave Hill, Jenolan; *S.* (*S.*) *tenax* (Fletcher, 1886), comb. nov. New South Wales: vicinity of Sydney.

Spenceriella (*S.*) *cormieri* species-group

Extramural calciferous glands absent.

(1) *S.* (*S.*) *cormieri*, sp. nov.* Queensland: Lamington Plateau.
(2) *S.* (*S.*) *curtisi*, sp. nov.* Queensland: Lamington Plateau.
(3) *S.* (*S.*) *minor* (Spencer, 1900), comb. nov.* Queensland: Cooran, Gayndah, Mt Tamborine, Blackall Range.
(4) *S.* (*S.*) *noctiluca*, Queensland: Lamington Plateau.
(5) *S.* (*S.*) *wiburdi* (Boardman, 1943). New South Wales: Mt George.

Subgenus **Austroscolex** Jamieson

Diagnosis

Gizzard rudimentary. Bucco-pharyngeal or other anterior nephridial tufts absent. Extramural calciferous glands absent.

Four species, see Jamieson (1977*b*).

Distribution

Lord Howe Island, Queensland, (Norfolk Island?, New Zealand?).

Spenceriella (Spenceriella) cormieri, sp. nov.*

(Figs 1, 21, 22; Table 1)

Description

Length of 105 mm (H), range 70–105, mean 84 mm, width (midclitellar) 2·4 (H), range 2·4–3·2, mean 2·9; segments 160 (H), range 120–169, mean 154 (H, P1–3). Approximately circular in cross-section; lacking appreciable secondary

Table 1. Intersetal distances in *Spenceriella (S.) cormieri*

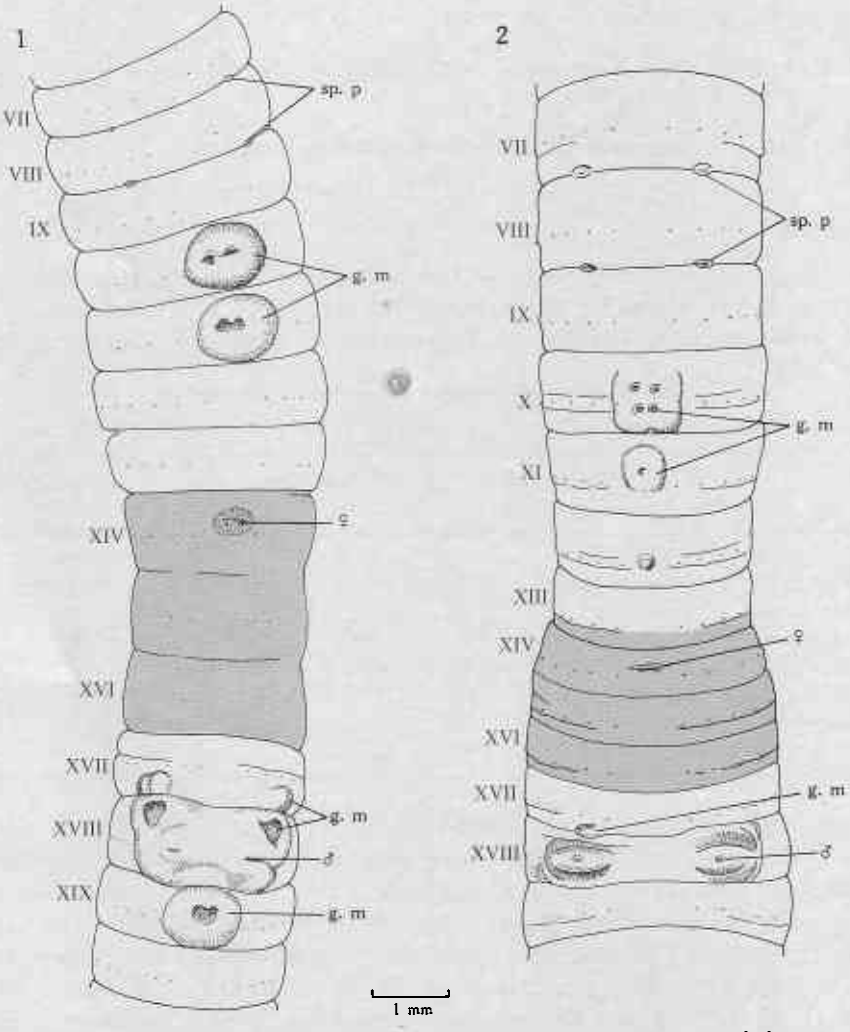
Values are for six specimens: the holotype, paratypes 1–4 and paratype 6

	Percentage of circumference					Circumference
	aa	*ab*	*bc*	*zy*	*zz*	(mm)
Segment XII						
Holotype	8·79	3·21	3·26	3·75	7·82	9·21
Minimum	7·72	2·68	3·15	2·81	5·49	
Maximum	11·30	4·09	4·69	4·59	8·38	
Mean	9·51	3·31	3·86	3·65	6·73	
Mean, interval/*ab*	2·89	1·00	1·18	1·12	2·04	
Segment XX						
Mean, interval/*ab*	3·31	1·00	1·19	1·11	2·06	

annulation. Reddish brown pigment dorsally, clitellum greyish yellow, in alcohol. Prostomium epilobous $\frac{1}{2}$ (H), $\frac{1}{3}$ (P1), open, dorsal tongue narrow, tapering posteriad. Peristomium bisected ventrally. First dorsal pore $\frac{4}{5}$ (H, P1). Setae commencing on II; numbers per segment 24 (H), range 24–28, mean 25, in XII; 28 (H), range 27–33, mean 29, in XX; 26 (H), range 26–34, mean 32, 15 segments from posterior end. In holotype locality, in XII, $aa : ab : bc : yz : zz = 2·9 : 1·0 : 1·2 : 1·1 : 2·0$ (mean H, P1–5); *a* and *b* lines straight throughout; *z* lines becoming irregular posteriorly (H, P1). Nephropores not externally visible. Clitellum annular, XIV–XVI, at full development strongly tumid but no wider than adjacent segments, dorsal pores obscured, setae and intersegmental furrows retained though fainter than elsewhere (H, P1). Transversely slit-like combined male and prostatic pores, a pair equatorially in *b* lines of XVII on low, poorly developed oval papillae which lie in a ventral tumid area which fills XVIII longitudinally, broadening anteriorly,

* Named for Professor Milton Cormier, Head of the Bioluminescence Laboratory, University of Georgia, in recognition of his encouragement of this research.

and which bears near the anterior border, in *bc*, anterolateral to the male pores a pair of glandular depressions; a pair of postsetal swellings preceding these at the posterior border of XVII; a circular midventral pad, with pore-like presetal depression, filling XIX longitudinally and including setae *a*; a similar pad in each of X and XI but the presetal depression or slit in each paired, median to *a* (H). Field variation (6 specimens): midventral pad with paired presetal depressions



Figs 1 and 2. Genital fields of: *1*, *Spenceriella* (*S.*) *cormieri*, sp. nov., holotype QM G8918; *2*, *Spenceriella* (*S.*) *curtisi*, sp. nov., holotype QM G8897.

in X (6; H, P1–5), XI (4; H, P1–3); a pair of posterior swellings in XVII (2; H, P2); a pair of depressions anterolateral to the male pores in XVIII (5; H, P1–4); a midventral pad with single presetal depressions in XIX (5; H, P1–4). Distance between male pores 1·02 mm (H), range 1·02–1·34, mean 1·26 (H, P1–5). Female pores an inconspicuous pair, in a common, oval glandular field, $< \frac{1}{3} aa$ apart, shortly presetal (H) or virtually equatorial (P1). Spermathecal pores 2 pairs, almost

concealed in intersegmental furrows 7/8 and 8/9, immediately lateral of *b* lines; 1·5 (P1), 1·6 (H) mm, 0·16 body circumference apart (H, P1).

Strongest septa 8/9–13/14, moderately strongly thickened, 12/13 the thickest. Last pharyngeal glands in V. Dorsal blood vessel single, continuous onto the pharynx. Last hearts in XII, those in X–XII latero-oesophageal, each receiving a broad, short blood-filled connective from the supra-oesophageal vessel and a very slender, colourless connective from the dorsal vessel (H, P1). Commissurals in VI–IX differing in being dorsoventral only and in sending each a branch to the body wall. Supra-oesophageal vessel well developed, in $\frac{1}{2}$VIII–$\frac{1}{2}$XIII (H, P1), a very slender continuation into VII visible in the better preserved P1. Subneural vessel absent.

Gizzard small, globular, muscular (though in P1 feebly) and easily compressible, in V, extending to the posterior border of VI by backward deflection of septa 5/6 and 6/7, which conceal it. Oesophagus with circumferential vascular striae in IX–XII; calciferous glands absent (H, P1). Intestine commencing in XVI, gradually (H) or abruptly (P1) widening; muscular thickening, caeca and typhlosole absent, though an insignificant dorsal ridge is present (H, P1).

Nephridia avesiculate micromeronephridia throughout; transverse bands in III posteriorly but a few in II; a pair of tufts in IV and V, those in V the larger, in IV enteronephric, a duct on each side passing to the anterior pharynx (H, P1); the ducts of those in V joining the buccal cavity well anterior to these (P1). Remaining nephridia of the oesophageal region forming parietal bands, thickest on the clitellum, in XV and XVI. Meronephridia of the intestinal region associated with the anterior septum of each segment (H, P1). Caudally forming very dense postseptal bands filling the segments longitudinally and joining the junction of septa and ventral body wall; a median preseptal funnel present, flanked by several post-septal intrasegmental funnels (H, P1); a postseptal duct running from the nephridia of a side to the intestinal wall beneath the dorsal blood vessel (P1).

Testes, large sperm masses and slightly iridescent sperm funnels free in X and XI; racemose seminal vesicles in IX and XII, approximately equisized, the two vasa eferentia of a side joining in XII (H, P1). Elongate ovaries consisting of several conjoined strings of large oocytes, and funnels, in XIII; smaller ovisacs in XIV. Prostates (tubulo?) racemose, the gland a broad, slightly depressed, posteriorly narrowing, marginally occasionally constricted but otherwise smooth lobe in XVIII and XIX, or XVIII only on the left (P1), the posterior end of the left gland reflexed (H); muscular duct forming a simple loop and joined at its junction with the gland by the vas deferens (H, P1). Spermathecae 2 pairs, discharging anteriorly in VIII and IX each with large spheroidal ampulla and well demarcated, moderately slender duct which is joined at its ental end by a strongly clubbed, inseminated diverticulum (H, P1). Length of right spermatheca of IX, 1·20 mm, ratio of total length : length duct, 3·0; ratio total length : length diverticulum, 1·0 (H).

Material Examined

Queensland: 28°16′S., 153°09′E. (approx.), Lamington National Park, in montane rain forest, in soil under leaf litter, B. G. M. Jamieson and R. J. Raven, 1.iv.1975, holotype (QM G8918), paratype 2 (QM G8919), paratype 3 (AM W6649), paratype 4 (BMNH 1979.1.3). Same locality in leaf litter at turning to monument near Mt. Bithongabel, Bruce Barnes, 20.ii.1978, paratype 1 (QM G8920), paratypes 5–7 (BJ 1975.4.1, 1978.5.18,19).

Remarks

Spenceriella (*S.*) *cormieri* is here designated the nominate species of a *cormieri* species-group within the subgenus *Spenceriella*, this species-group being distinguished from the typical species-group in lacking extramural calciferous glands. It is distinguished from three other members of the *cormieri* group, (*S.* (*S.*) *minor*, *noctiluca* and *wiburdi*) by its setal ratios and by its distinctive genital fields. The fifth member of the *cormieri* group, *S.* (*S.*) *curtisi*, is morphologically close, and its setal ratios are not significantly different. Of the many species in which setal ratios have been measured, transgression of species limits by the same population of ratios has elsewhere been recorded only between *Fletcherodrilus unicus* and *F. fasciatus* (this account). The genital field of *S.* (*S.*) *cormieri* distinguishes it from *S.* (*S.*) *curtisi*, notably in the paired presetal depressions (on median pads) in X and XI, the pair of depressions anterolateral to the male pores in XVIII. and the midventral pad, with depression, in XIX. The luminescence of *S.* (*S.*) *cormieri* is also significantly brighter than that of *S.* (*S.*) *curtisi*.

The similarity of the setal ratios between the two entities, general anatomical similarity, and resemblances of the genital fields, notably the shared presence in at least some specimens of posterior paired pads in XVII, indicate that the two are more closely related to each other than to other species of *Spenceriella*. Differences are taken to indicate that reproductive isolation has occurred, but if so it is probable that speciation took place relatively recently on the Lamington Plateau in response to microgeographic and ecological factors. Further study of this problem is in progress.

Luminescence of S. (S.) cormieri

The bioluminescence of *S.* (*S.*) *cormieri* is very bright and easily visible to the non-dark-adapted eye. Three specimens were examined quantitatively, with the range of luminescence in the exudate from $0{\cdot}28 \times 10^{11}$ to 2×10^{11} photons per second. Emission from a buffer suspension of the coelomic fluid was slightly stimulated by addition of distilled water but no other evidence for a particulate bioluminescence system was obtained. Addition of dilute hydrogen peroxide solution to a buffer extract of the fluid caused more than a 100-fold increase in the luminescence intensity. Subsequent addition of *Diplocardia* luciferase stimulated it only slightly. When *Diplocardia* luciferin was added after peroxide, a twofold increase in luminescence followed the addition of luciferin.

Spenceriella (Spenceriella) curtisi, sp. nov.*

(Figs 2–5, 23, 24; Table 2)

Population (a): Typical Population

Description

Length 65, 61 mm, width (midclitellar) 3·1, 3·4 mm, segments 111, 108 (H, P1). Approximately circular in cross-section. Lacking secondary annulation. Pigmented purplish brown dorsally; clitellum brick red. Prostomium epilobous $\frac{1}{2}$, open, slightly

* Named for Mr S. Curtis, Department of National Parks and Wildlife, Brisbane, who by his kind cooperation greatly facilitated this study.

tapering; dorsal tongue moderately wide, almost $\frac{1}{3}$ the width of the peristomium. Peristomium bisected ventrally. First dorsal pore 4/5 imperforate (P1), 5/6 perforate (H, P1). Setae commencing in II; numbers per segment 20 (H), range 18–20, mean 19 (H,7 paratypes) in XII;119–24, mean 21 (7 paratypes) in XX; 30 (H), range 23–30, mean 25 (H,7 paratypes) at 15 segments from posterior end. In holotype locality, in XII, $aa : ab : bc : yz : zz = 2{\cdot}2 : 1{\cdot}0 : 1{\cdot}1 : 1{\cdot}3 : 1{\cdot}6$ (mean H, P3); *a*, *b* and *z* lines straight throughout. Nephropores not externally visible. Clitellum annular, $\frac{1}{2}$XIII (H), XIV (P1)–XVI (H, P1); at full development (P1) strongly tumid though only slightly wider than adjacent segments; dorsal pores obliterated; intersegments distinct only ventrally; setae retained. Small slit-like combined male and prostatic pores (each in H with narrow lips), a pair in *b* lines of XVIII on large transversely oval papillae; each papilla surrounded by a dark translucent moat (H) or elevated rim (P1); the pores separated by 1·84 mm (H), range 1·25–1·89,

Table 2. Intersetal distances in *Spenceriella (S.) curtisi*

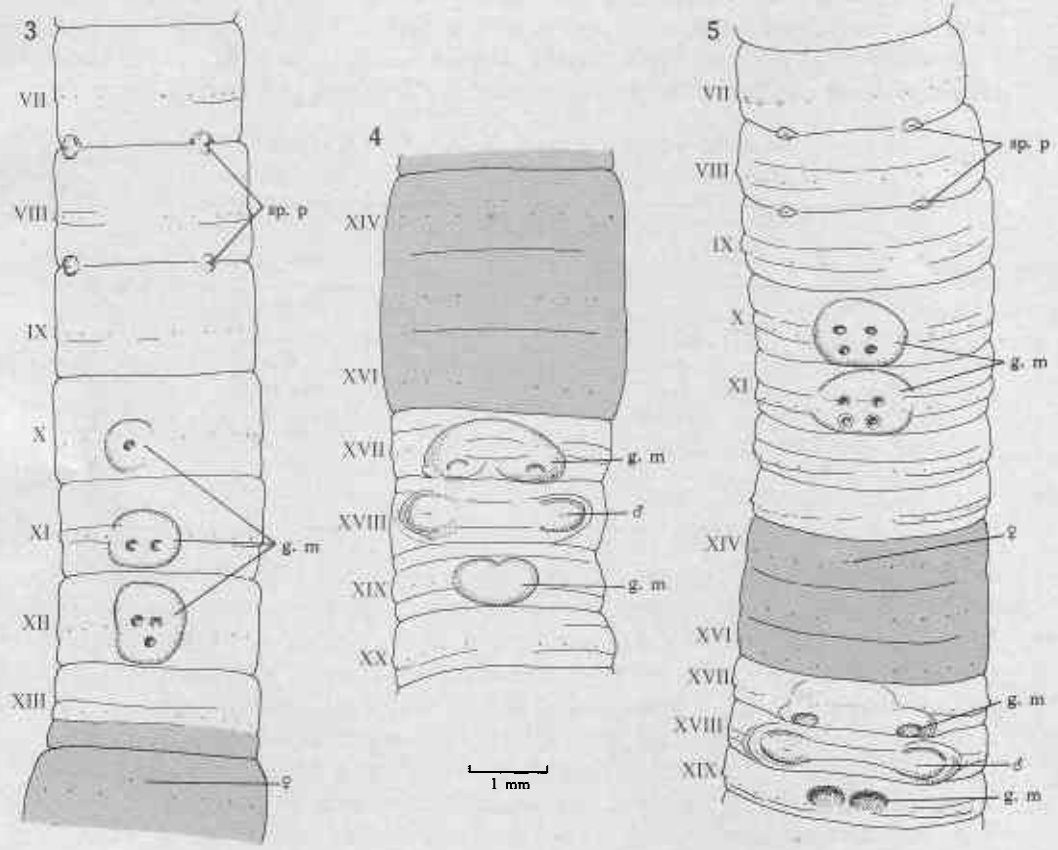
Values are for eight specimens: the holotype and paratypes 1–7

	Percentage of circumference					Circumference
	aa	*ab*	*bc*	*zy*	*zz*	(mm)
Segment XII						
Holotype	10·23	4·04	4·18	5·41	7·06	6·94
Minimum	6·98	2·72	3·11	3·75	4·48	
Maximum	10·90	4·39	5·86	5·87	8·89	
Mean	8·99	3·90	4·39	4·86	6·55	
Mean. interval/*ab*	2·30	1·00	1·13	1·26	1·68	
Segment XX						
Mean. interval/*ab*	2·31	1·00	1·08	1·12	1·55	

mean 1·68 mm; 0·17 body circumference apart (H), range 0·11–0·19, mean 0·16 (H, 7 paratypes). Accessory genital markings in 2 Tamborine specimens, a square midventral pad in X bearing a pair of presetal and a pair of postsetal dimplelike markings, forming a tetrad; a midventral circular pad in XI with single, median, presetal dimple (H, P3); a small, paired (P3) or unilateral, right (H) elliptical pad at the posterior border of XVII in *ab*; a small hemispheroidal papilla in the setal arc of XII (H only) questionably a genital marking. In 18 clitellate paratypes from Lamington accessory genital makings consist usually of a tetrad of dimples on X, somewhat less commonly on XI, infrequently on XII and, rarely, a postsetal pair on IX: in some specimens the tetrad on X or XI is replaced by a triad of which 2 of the dimples are presetal; other, infrequent variants are given in the following detailed account of the disposition of markings (numbers of specimens in parentheses).

A pair of dimples median to *a* and postsetal in IX (1; P15); presetal in X (9; P1,2,4–6,8,9,12,15); postsetal in X (16; P1,2,4–9,11–16,18,19 right); presetal in XI (15; P1,2,4–15,17,18); postsetal in XI (15; P1,2,4–10,13–15,17–19); presetal in XII (7; P2,5,8–10,18,19); postsetal in XII (3; P2,8,9); a single median dimple postsetal in X (1; P17) XI (1; P11); or XII (5; P8,10,13,18,19); presetal in XII (2; P5,13) or left equatorial in XI (1; P16). A pair of elliptical pads at the posterior margin of XVII in *ab* (16; P1,2,4–17); a pair of glandular depressions presetally

and median to *a* of XIX (11; P1,2,4–6,12,14,15,17–18); a pair of glandular depressions median of *a* postsetally in XIX (1; P16) or a median heart-shaped pad in the posterior $\frac{2}{3}$ of XIX (1; P19). Paratypes 28–30 have a pair of presetal dimples in each of VII and VIII in addition to the tetrad in X. Female pores a small pair, close together medianly, shortly presetal in XIV. Spermathecal pores 2 pairs, in 7/8 and 8/9, immediately lateral of *b* lines, clearly visible minute pores with lips forming an elliptical papilla (H, P1); the pores separated by 1·56 mm (H), range 1·36–1·79, mean 1·63 (H, 7 paratypes), 0·17 (H), range, 0·16–0·22, mean 0·18 body circumference (H, 7 paratypes).



Figs 3–5. Genital fields of *Spenceriella* (*S.*) *curtisi*, sp. nov.: *3*, paratype 19 QM G1890, anterior field; *4*, paratype 19 QM G8910, posterior field; *5*, paratype 1 BMNH 1978.1.14.

Strongest septa 7/8–12/13, moderately strongly thickened. Last pharyngeal glands in V; no separate septal glands evident. Dorsal blood vessel single, continuous onto the pharynx. Last hearts in XII, those in X–XII latero-oesophageal, each receiving a broad short connective from the supra-oesophageal vessel and a slender colourless connective from the dorsal vessel. Commissurals in (VII?) VIII–IX dorsoventral only and differing from those in X–XII in giving off a branch to the body wall before joining the ventral vessel. Supra-oesophageal vessel VIII–$\frac{1}{2}$XIII. Subneural vessel absent (H, P3). Suboesophageal vessel in IX bifurcating

in VIII to give a pair of large latero-oesophageal vessels running forwards median to the commissurals (P3).

Gizzard moderately large and firm, but not strong, barrel-shaped, in V, ensheathed in septum 5/6, reaching ½VIII by backward deflection of septa. Oesophagus with circumferential vascular striae in IX–XV; calciferous glands absent. Intestine commencing in XVI; muscular thickening, caeca and typhlosole absent.

Nephridia avesiculate meronephridia throughout. Bands of exonephric, astomate meronephridia in II–VII. A pair of large tufts in V sending ducts to the dorsolateral aspect of the buccal cavity in III (H, P1), tufts in VI, shown in P1 to send ducts to the buccal cavity ventrally in III. Some intrasegmental nephrostomes demonstrable in the oesophageal region (e.g. VIII and IX) (H, P1). Nephridia strewn on the parieties in IX–XIII; dense bands in XIV–XVII; thereafter nephridia in 2 longitudinal zones on each side. Caudally with numerous (enteronephric?) tubules on the anterior septa. A few penultimate caudal segments with nephridia forming clusters on which, intrasegmental nephrostomes are visible; a median preseptal funnel present.

Testes and iridescent sperm funnels free in X and XI; racemose, elongate seminal vesicles in IX and XII, the anterior pair the larger. Wisp-like ovaries, with several strings of large oocytes, and funnels in XIII; ovisacs in XIV. Prostates tubuloracemose (?, no central canal detected in hand sections), S-shaped in XVIII and XIX; the long once-looped muscular duct widening to the pore and receiving the vas deferens near its ental end. Spermathecae 2 pairs, discharging anteriorly in VIII and IX; each with broad sacciform ampulla and well demarcated moderately slender, muscular duct which is joined near its ental end by a digitiform, apically somewhat clubbed, inseminated diverticulum (H, P1). Length of left spermatheca of IX, 1·28 mm; ratio of total length : length duct, 2·0; ratio total length : length diverticulum, 1·1 (H).

Material Examined

Queensland: 27°55′S., 153°10′E., Mt Tamborine, Palm Grove National Park, in clayey brown loam under leaf litter in dense palm forest with some *Ficus* trees, J. E. Wampler and B. G. M. Jamieson, 2.ii.1978, holotype and paratype 3 (QM G8897–8898). 28°16′S., 153°09′E. (approx.), Lamington National Park, in montane rain forest, *c.* 2 km from O'Reilly's Guest House, in soil under leaf litter, J. E. Wampler and B. G. M. Jamieson, 2.ii.1978, paratypes 1 and 2 (BMNH 1978.1.14,15), paratypes 4–7 (AM W6639–6642). Same locality, *c.* 1 km from O'Reilly's Guest House, B. M. Barnes, 13.ii.1978, paratypes 8–19 (QM G8899–8910), paratypes 20–27 (BJ 1978.2.1–8), paratypes 31–38 (BJ 1978.5.8–15). Same locality, *c.* 5 km from O'Reilly's Guest House, in *Nothofagus* forest, B. M. Barnes, 20.ii.1978, paratypes 28–30 (BJ 1978.5.5–7), paratype 39 (BJ 1978.5.17).

Remarks

The tetrad of pore-like genital markings in X in *S.* (*S.*) *curtisi* is reminiscent of the condition in *S.* (*S.*) *tenax*, *S.* (*S.*) *jenolanensis* and *S.* (*S.*) *wiburdi* and some other species still included in *Megascolex*. However, in those species the pore-like depressions are situated laterally to or very shortly median to setae *a*, not, as in *S.* (*S.*) *curtisi*, far median to them. Also *S.* (*S.*) *tenax* and *S.* (*S.*) *jenolanensis* differ from *curtisi* in possessing calciferous glands (*notabilis* group). Resemblance of the genital field in *wiburdi* to that of *cormieri* appears superficial, and examination of the syntypes of *wiburdi* reveals significantly different setal ratios (Wilcoxon test for 5% probability).

Population (b)

A small sample of Lamington worms collected slightly apart from samples of the typical population, and consisting of semimature and mature specimens, shows at full maturity a consistently different field. The major difference is the constant absence of posterior genital pads in XVII. In some individuals these are replaced by a pair of markings anteromedian to the male pores, in XVIII. Otherwise the genital field is as in the typical population, with a tetrad on X and a presetal pair of dimples in XI. A presetal pair of dimples, in VII and VIII, rare in the typical population, is usual. It will be seen that luminescence in these specimens is an order of magnitude greater than in the typical population. These specimens will not be designated paratypes, pending further investigation of the complex.

Material Examined

Queensland: 28°15′S., 153°09′E. (approx.) Lamington National Park, in montane rain forest, *c.* 2·5 km from O'Reilly's Guest House, in soil under leaf litter, B. M. Barnes, 20.ii.1978, specimens 1–9 (BJ 1978.5.23–31).

Luminescence of S. (S.) curtisi

S. (S.) curtisi is moderately to weakly luminescent, requiring full dark adaptation to be visible. Of the 21 specimens of population A examined quantitatively, the emission levels from the exuded fluid ranged from undetectable to 1×10^9 hv s^{-1} with a mean peak intensity of 2×10^8 photons s^{-1}. Interestingly, the small sample of population *b* (nine specimens) had consistently brighter luminescence (mean 2×10^9, range $0{\cdot}6–5 \times 10^9$ photons s^{-1}).

When worms were stimulated in buffer, no luminescence was seen and the resulting suspension of coelomic fluid was easily separated by centrifugation into a soluble and a particulate fraction. The particulate material was resuspended and washed twice with buffer, then centrifuged. When the particles were resuspended, luminescence was seen on addition of water; strong stimulation by dilute peroxide was also seen. The original supernate from the initial centrifugation had a no luminescence activity.

Extracts of the exuded fluid and separated particles were stimulated 10–20 times upon addition of dilute peroxide. Subsequent addition of *Diplocardia* luciferase did not usually result in further stimulation. Similarly, *Diplocardia* luciferin, when added to solutions previously stimulated by peroxide addition, had little or no effect. In the case of paratype 1 (BMNH 1978. 1.14), where no luminescence was detected in the exuded fluid, addition of peroxide gave a very weak emission (2×10^7 photons s^{-1}), but when *Diplocardia* luciferase was added a 50-fold increase in intensity was seen.

Spenceriella (S.) minor (Spencer)

(Figs 8, 25, 26; Table 3)

Megascolex minor Spencer, 1900, pp. 49–50, pl. 8, figs 55–57; Michaelsen, 1916, p. 27; Sweet, 1900, pp. 119–29; Jensz and Smith, 1969, p. 98.

Description

This account refers to Tamborine specimens 1 and 2 (S1, S2) unless otherwise stated.

Length 31 mm (S2), width (midclitellar) 1·1 (S2)–1·3 mm (S1), segments 94 (S2; S1 is posterior amputee). Approximately circular in cross-section. Pigmentless. Prostomium epilobous $\frac{1}{2}$, open, narrow, parallel-sided; peristomium bisected ventrally. First dorsal pore 4/5. Setae commencing on II; *a*, *b* and *z* lines straight; in XII $aa : ab : bc : zy : zz = 1{\cdot}8 : 1{\cdot}0 : 0{\cdot}9 : 1{\cdot}0 : 1{\cdot}6$ (mean of 3 Tamborine). Nephropores not externally visible. Clitellum annular, moderately protuberant, strongly delimited at XIV–XVI (S1, S2) but some clitellar modification from $\frac{1}{2}$ XIII to $\frac{1}{3}$ XVII (S1). Minute transversely slit-like combined male and prostatic pores

Table 3. Intersetal distances in *Spenceriella (S.) minor*

From Tamborine, specimens 2, 4 and 5; from Lamington, specimens 6–9

	Percentage of circumference					Circumference
	aa	*ab*	*bc*	*zy*	*zz*	(mm)
Tamborine						
Segment XII						
Specimen 2	5·85	4·62	4·46	5·24	7·38	3·25
Specimen 4	10·00	5·30	3·97	5·74	9·41	3·40
Specimen 5	10·76	4·73	4·73	3·94	6·30	3·81
Mean	8·87	4·88	4·39	4·97	7·70	3·49
Mean, interval/*ab*	1·81	1·00	0·91	1·02	1·57	
Segment XX						
Mean, interval/*ab*[A]	2·09	1·00	0·80	0·94	1·28	
Lamington						
Segment XII						
Specimen 6	9·42	4·08	3·40	5·35	5·62	5·52
Specimen 7	9·23	3·69	3·97	4·15	6·27	5·42
Specimen 8	8·53	3·47	4·00	4·26	8·53	5·63
Specimen 9	8·32	3·18	3·41	3·10	6·20	6·61
Mean	8·88	3·61	3·70	4·22	6·66	5·80
Mean, interval/*ab*	2·47	1·00	1·15	1·31	2·46	
Segment XX						
Mean, interval/*ab*	2·49	1·00	0·96	1·01	1·67	
Tamborine and Lamington						
Segment XII						
Minimum	5·85	3·02	3·53	2·55	5·11	
Maximum	10·76	5·59	5·23	6·47	9·41	
Mean	8·86	4·16	4·21	4·54	6·95	
Mean, interval/*ab*	2·18	1·00	1·04	1·10	1·69	
Segment XX						
Minimum	8·03	2·95	2·81	3·21	4·55	
Maximum	10·98	5·42	4·26	5·49	10·59	
Mean[B]	9·51	4·12	3·64	3·95	6·33	
Mean, interval/*ab*	2·36	1·00	0·90	0·99	1·54	

[A]S2 and S4 only. [B]S2, S4 and S6–S9 only.

a pair on XVIII, in *ab*, on large but poorly developed transversely oval papillae; the pores 0·74, 0·72 mm, 0·20, 0·16 body circumference apart (S1, S2). Accessory genital markings (examined in 4 Tamborine specimens): a midventral transverse pad in XI (3 specimens, absent from S4), XX (4 specimens) and XXI (1 specimen only), filling the segment longitudinally, extending laterally of *b*, those in XI and XXI each with a pair of presetal pore-like markings, almost contiguous medianly

of *a*; that in XX with a single, median presetal pore-like marking. A trough-like depression postsetally in XVII (S1 only) and presetally in XIX, with crescentic borders convex anteriorly and posteriorly, respectively (S1 only); that in XIX with a pair of pore-like markings within its lateral limits (these pore-like markings present in S3,S4 in the absence of depressions). A pore-like marking also present medianly between the male porophores (S1, S3). A pair of pore-like markings anterolateral of the male pores, near the anterior border of XVIII (S4). Four Lamington specimens tend to have more extensive genital markings. Pore-like markings on the transverse midventral pads are single or, usually paired; segmental distribution of markings (numbers of specimens in parentheses) is as follows: X(2), XI(4), XII(4), XIII(2), XX(4), XXI(4), XXII(1). Female pores an inconspicuous pair shortly presetal, but almost contiguous medianly, in XIV (S1, S2). Spermathecal pores minute (demonstrable only by parting adjacent segments), 2 pairs, in 7/8 and 8/9, in *b* lines (S1) or immediately lateral of these (S2); the posterior pair approximately 0·76 mm, 0·17 body circumference apart (S1).

Thickest septa 9/10 and 10/11(S1) or 11/12 and 12/13 (S2) but none strong. Dorsal blood vessel single, continuous onto the pharynx. Last hearts in XII, those in X–XII latero-oesophageal, each receiving a broad, short connective from the supra-oesophageal vessel. Supra-oesophageal vessel in $\frac{1}{2}$VIII–$\frac{1}{2}$XIII. Subneural vessel absent.

Gizzard moderately large and moderately strongly muscular in V but reaching to posterior VII by external segmentation. Oesophagus vascular in VIII–XV, less so in XVI in which it narrows before commencement of the intestine at $\frac{1}{2}$XVI; muscular thickening, caeca and typhlosole absent.

Nephridia avesiculate micromeronephridia throughout; a pair of large (enteronephric?) tufts present at the anterior limit of the gizzard in V and VI. Caudal nephridia: median preseptal funnel present; median nephridium enlarged; intrasegmental funnels not detectable with certainty. Testes, sperm masses and large iridescent sperm funnels free in X and XI; racemose seminal vesicles in IX and XII, the posterior the larger. Ovaries, with several strings of large oocytes, and female funnels in XIII. Large ovisacs with several loculi which correspond in size with a mature ovarian oocyte dependent from the anterior wall of XIV. Prostates tubuloracemose?, the glandular portion in XVIII and XIX, flattened, S-shaped, a long, slender, curved but not tortuous muscular duct arising from the anterior end. Vas deferens joining the gland a short distance from the duct. Penial setae absent. Spermathecae 2 pairs, discharging anteriorly in VIII and IX, each with an ovoid ampulla imperceptibly grading into a tapering duct which is joined entally by a lateral clavate inseminated diverticulum; size of spermathecae approximately uniform; length of right spermatheca of IX (S1), 0·72 mm; ratio of total length : length duct, 1·9; ratio total length : length diverticulum, 1·4.

Material Examined

Queensland: 27°55′S., 153°10′E., Mt Tamborine, Palm Grove National Park, in clayey brown loam under leaf litter in dense palm forest with some *Ficus* trees, J. E. Wampler and B. G. M. Jamieson, 21.i.1978, specimen 1 (BJ 1978.5.20); same data, 29.i.1978, specimen 2 (QM G8921); specimen 3 (BMNH 1979.1.4); specimens 4 and 5 (BJ 1978.5.21,22). 25°16′S., 153°09′E. (approx.), Lamington National Park, in montane rain forest, in soil under leaf litter, B. M. Barnes, 20.ii.1978, specimens 6–9 (QM G8927–8930).

Previous Records

Cooran and Gayndah (type localities). Mt Tamborine and Blackall Range (Michaelsen 1916).

Remarks

Michaelsen (1916) draws attention to an error with regard to the location of genital markings, in Spencer's type description of *Megascolex minor*. It appears that markings were in X, XI and XX. Whether the specimens from Mt Tamborine described by Michaelsen and in this account are assignable to *S.* (*S.*) *minor* is not entirely certain. Michaelsen observed anterior markings (presumably in X and XI) and a well developed marking on XX in some Tamborine specimens. Some of Michaelsen's specimens had additional markings in XIX, as does one of the present Tamborine specimens. Markings are usually present in XI and XX in the latter, and absence from X would not seem sufficient to separate them from Michaelsen's sympatric material. In the Lamington specimens markings are present or absent on X but are constant in XI and XX (as on XII and XXI). None of Michaelsen's specimens in the Hamburg Museum (HM V8466; examined by B.G.M.J.) has genital markings apart from a weak anteriorly convex crescentic marking in XVII in one specimen, but conspecificity with the new material is acceptable. Agreement of Tamborine and Lamington material with Spencer's description and illustration of *M. minor* is sufficiently close to suggest conspecificity, but an attempt to confirm this by reference to the types has failed. Specimens in the National Museum of Victoria from Cooran, which Jensz and Smith (1969) reasonably considered syntypes of *M. minor* because they bore Spencer's manuscript code (Peri sp 3Q) for this species have no markings in X, XI or XX (one has a transverse row of pore-like markings in VIII) but some have a pair of large presetal markings in XIX. Body size is larger and, although their setal ratios are not significantly different from the Mt Tamborine specimens by the Wilcoxon test, it is doubtful that they are conspecific with the material described in the type-description. Attempts to collect new material of the species from the type localities have been unsuccessful.

Luminescence of S. (S.) minor

S. (*S.*) *minor* is moderately bright, being easily visible to the dark-adapted eye. The peak intensity of bioluminescence of nine specimens ranged from 2×10^8 photons s^{-1} to 2×10^{10} photons s^{-1}. No data were obtained relative to the particulate nature of the luminescence system.

Extracts of the exuded coelomic fluid showed several-fold stimulation (1–6×) by addition of dilute peroxide, slight stimulation (2×) by subsequent addition of *Diplocardia* luciferase, and variable but significant stimulation by *Diplocardia* luciferin.

Spenceriella (Spenceriella) noctiluca, sp. nov.

(Figs 6, 7, 9, 27, 28; Table 4)

Description

Length 40–88, mean 60·6 mm (10 paratypes); width (midclitellar) 3·0 (H), 2·1–3·0, mean 2·6 mm (H, 9 paratypes). Segments 100–127, mean 116 (holotype is posterior amputee). Approximately circular in cross-section. Lacking appreciable

secondary annulation. Reddish pigment dorsally. Prostomium epilobous $\frac{1}{2}$, narrow, parallel-sided; peristomium bisected ventrally (H, 10 paratypes). First dorsal pore 4/5, imperforate (H, P5), 5/6 perforate (8 paratypes). Setae commencing in II; numbers per segment 24 (H), range 24–25, mean 24 (H, 9 paratypes), in XII; 24 (H), range 24–28, mean 26 (H, 9 paratypes) in XX; 23–28, mean 25 (9 paratypes), 15 segments from posterior end. In holotype locality, in XII, *aa* : *ab* : *bc* : *yz* : *zz* averaging 2·9 : 1·0 : 1·3 : 1·2 : 3·0 (H, 6 paratypes); *a*, *b* and *z* lines straight throughout, though with some irregularity which increases near the caudal extremity. Nephropores not externally visible. Clitellum annular, strongly protruberant at full development (H), $\frac{1}{2}$XIII (H, 4 paratypes), XIV (2 paratypes)–XVI (2 paratypes), $\frac{1}{3}$XVII (H, 4 paratypes), 3–3 $\frac{5}{8}$ segments. Minute combined male and prostatic pores a pair on XVIII, in *ab* (H, 6 paratypes) or *b* (3 paratypes), on small oval papillae each of which is situated on a low mound-like porophore which medially tapers narrowly towards that of the other side leaving a small intervening

Table 4. Intersetal distances in *Spenceriella (S.) noctiluca*

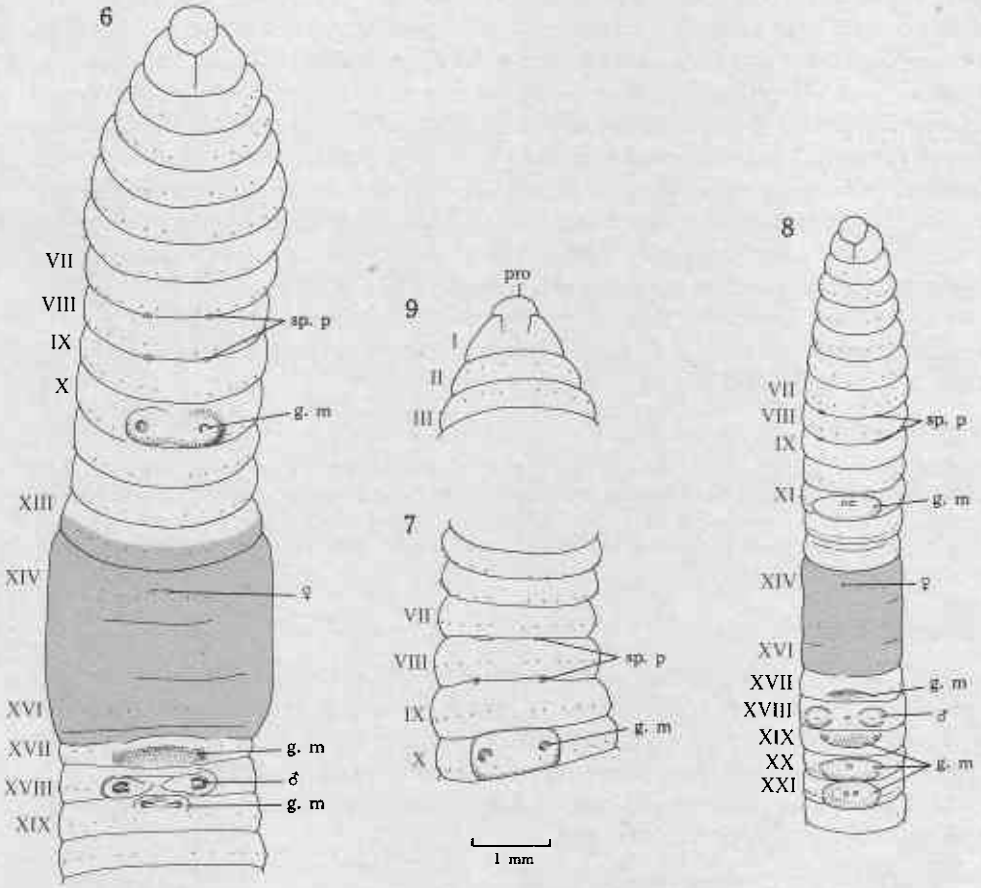
Values are for seven specimens: the holotype and paratypes 1–6

	Percentage of circumference					Circumference (mm)
	aa	*ab*	*bc*	*zy*	*zz*	
Segment XII						
Holotype	8·57	6·36	3·25	2·52	8·57	6·77
Minimum	6·12	2·47	3·25	2·22	7·65	
Maximum	9·80	5·07	5·29	4·64	9·87	
Mean	8·35	3·07	3·96	3·53	8·85	
Mean, interval/*ab*	2·86	1·00	1·34	1·21	3·01	
Segment XX						
Mean, interval/*ab*	3·68	1·00	1·45	1·31	2·75	

gap; the pores separated by 1·0 mm, range 0·71–1·04, mean 0·90 mm (H, 8 paratypes); 0·12 body circumference apart (H), range 0·09–0·13, mean 0·12 (H, 8 paratypes). Accessory genital markings: a pair of medianly conjoined prominences in X, each with a presetal porelike centre in *ab* (H, 9 paratypes), unilateral, left in 1 paratype. An unpaired, transverse median pad in each of XVII and XIX, respectively with a postsetal and presetal glandular trough. Female pores a pair anteromedian of *a* of XIV. Spermathecal pores inconspicuous (demonstrable only by parting adjacent segments), 2 pairs in 7/8 and 8/9, in (H, 7 paratypes) or very slightly lateral of *b* lines (3 paratypes); the posterior pair 0·72, 0·88 mm, 0·10, 0·12 body circumference apart (H, P3).

Thickest septa 7/8 and 8/9 (H) 12/13 and 13/14 (P1), moderately strongly thickened. Last septal glands paired in VI, large masses attached to the posterior septum (H). Dorsal blood vessel single, continuous onto the pharynx. Last hearts in XII, those in X–XII latero-oesophageal, each receiving a broad, short connective from the supra-oesophageal vessel (H, P1) the connective from the dorsal vessel demonstrated in X (P1). Commissurals in VI–IX dorsoventral only. Supra-oesophageal vessel in $\frac{1}{2}$VIII–$\frac{1}{2}$XIII. Subneural vessel absent. A pair of large latero-parietal vessels originating from the suboesophageal vessel in XIII and extending posteriorly to the vicinity of the prostate glands.

Gizzard moderately large, broad and short, readily compressible, in V, preceded in IV by a muscular, but not glossy, slightly smaller proventriculus. Oesophagus moniliform, with intersegmental constriction, in VI–XV; not evidently vascularized in VI and VII, thereafter with circumferential vascular striae, especially in XI–XIII (P1), XIV (H), in which it is internally rugose though extramural calciferous glands are absent; in XV (H) or XIV and XV (P1) differing in possessing a chloragogenous covering. Intestine commencing in XVI, muscular thickening, caeca and typhlosole absent.



Figs 6–9. Genital fields of: *6*, *Spenceriella* (*S.*) *noctiluca*, sp. nov., holotype QM G8922; *7*, *Spenceriella* (*S.*) *noctiluca*, sp. nov., paratype 3, anterior field; *8*, *Spenceriella* (*S.*) *minor* (Spencer, 1900), specimen 1, Tamborine; *9*, *Spenceriella* (*S.*) *noctiluca*, sp. nov., paratype 3, dorsal view of prostomium and first three segments.

Nephridia avesiculate meronephridia throughout, forming a dense apparently exonephric cluster on each side in II and III; a large tuft in IV with numerous long loops and a composite duct which passes to the roof of the pharynx at its junction with the buccal cavity (H). Whether this tuft belongs to IV or V is uncertain; in paratype 1 its tubules are in contact with the gizzard, in V. Clusters in the following 2 segments are diffuse, appear exonephric, and are transitional to parietal bands of meronephridia in subsequent segments, these bands being

especially dense in XIV, XV and XVI, the clitellar segments. No funnels certainly detectable in anterior intestinal and preceding segments. Caudally at least the medianmost nephridium has a preseptal funnel and this (enteronephric?) nephridium is enlarged, approaching the megameronephridial condition; at least some of the nephridia lateral to this have single intrasegmental nephrostomes (P1,2,4).

Testes, sperm masses and large iridescent sperm funnels free in X and XI; racemose seminal vesicles in IX and XII, the posterior pair the larger. Ovaries, bushy with many conjoined strings of large oocytes, and female funnels in XIII. Large ovisacs with numerous loculi, each corresponding in size to a mature ovarian oocyte, dependent from the anterior wall of XIV. Prostates racemose, the glandular portion in XVII–XIX; flattened, S-shaped but with the anterior 2 limbs united and jointly giving rise to the long, once-looped muscular duct. Vas deferens joining the prostate duct at its junction with the gland. Penial setae absent. Spermathecae 2 pairs, discharging anteriorly in VIII and IX, each with an ovoid, sometimes slightly bilobed ampulla with short, broad poorly demarcated duct which is joined laterally by an almost equally long, thickly digitiform to clavate inseminated diverticulum. Sperm free in the lumen of the diverticulum but grouped into numerous cylindrical bundles. Size of spermathecae approximately uniform (H,P1); length of left spermatheca of IX (H), 1·2 mm: ratio total length : length duct, 5·8; ratio total length : length diverticulum, 1·49.

Material Examined

Queensland: 28°19′S., 153°05′E. (approx.), Lamington National Park, in montane rain forest, *c.* 2 km from O'Reilly's Guest House in rotting log moistened by (fungal?) decomposition, in otherwise dry conditions. J. E. Wampler and B. G. M. Jamieson, 14.i.1978; holotype, paratypes 1–4 (QM G8922–8926); paratypes 5 and 6 (AM W6650–6651), paratypes 7 and 8 (BMNH 1979.1.5.6); paratypes 9 and 10 (BJ 1978.5.32,33).

Remarks

The distribution of genital markings distinguishes *S.* (*S.*) *noctiluca* from other species of the *cormieri* species-group to which it belongs. Its setal ratios provide a reliable means of recognition and separation from the sympatric *S.* (*S.*) *cormieri* and *S.* (*S.*) *curtisi*, complementing use of the genital field for diagnosis.

This species has been found only in rotting logs, but that it is restricted to this habitat requires further confirmation.

Luminescence of Spenceriella (S.) noctiluca, *sp. nov.*

S. (*S.*) *noctiluca* is moderately bright ($\sim 10^{10}$ photons s^{-1}). When worms are shocked in buffer, the particles in the exuded fluid are easily separated by centrifugation. Following washing as described for *S.* (*S.*) *curtisi*, the particle fraction was stimulated to emit by addition of distilled water; it was strongly stimulated by dilute peroxide and also by subsequent addition of *Diplocardia* luciferase (2×). The resuspended particle fraction was examined microscopically. The major components of this fraction were spherical coelomic cells packed with granular particles. Both the size and general morphology of these cells were analogous to the free chloragagen cells which are the site of luminescence in *D. longa* (Rudie and Wampler 1978).

Tribe **PERIONYCHINI** Jamieson

Genus **Fletcherodrilus** Michaelsen, emend.

Diagnosis

Medium to large terrestrial worms (85–325 mm) with < 180 segments. With strong purplish to brown parietal pigmentation. Prostomium slightly epilobous to epitanylobous. First dorsal pore 4/5 or 5/6. Setae 8 to numerous in setigerous segments; if 8, setae *c* and *d* distant, not paired. Penial setae absent. Nephropores in *d* lines or (*sigillatus*) in a sinuous line. Clitellum annular, occupying 4–5 segments, beginning in XIII or XIV. The combined opening of the male and prostatic pore unpaired, midventral. Accessory genital markings absent. Female pores anteromedian to setae *a* of XIV, inconspicuous. 3 or 5 unpaired, midventral spermathecal pores, ending at 8/9.

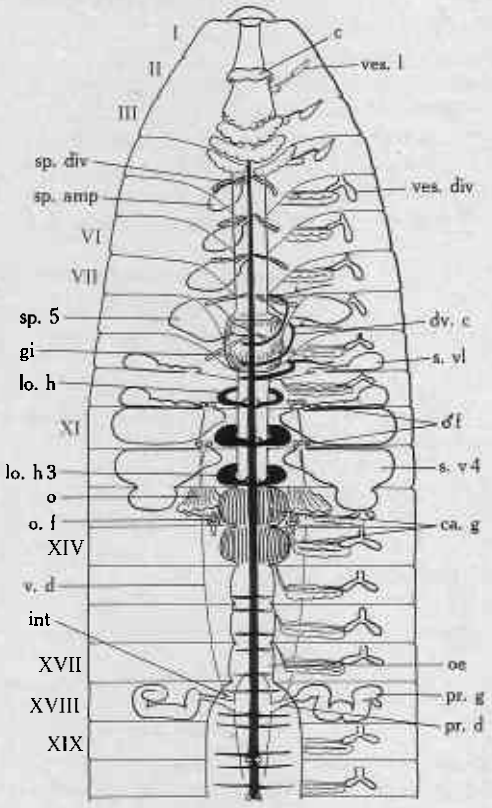
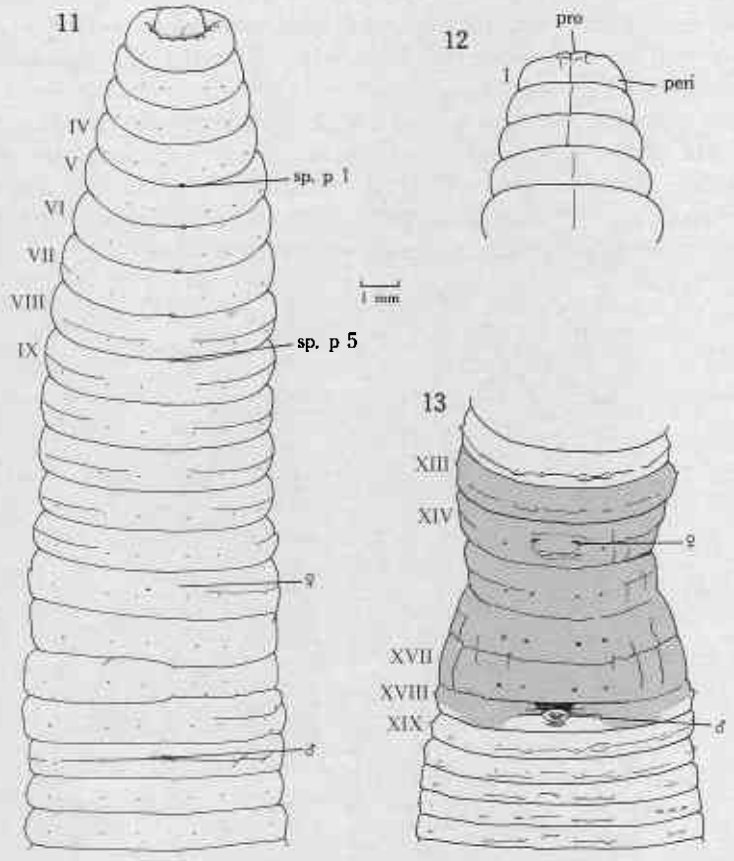


Fig. 10. Diagram of the internal anatomy of *Fletcherodrilus fasciatus* (Fletcher, 1890), dorsal view (based on specimen 1, Binnaburra).

Some preclitellar septa thickened. Gizzard well developed, in VI or VII. Calciferous glands lateral, sessile pouches in XIII and XIV or XIII, XIV and XV, with internal laminae but not constricted off from the oesophagus. Intestine commencing in XVIII; typhlosole and caeca absent. Dorsal blood vessel continuous onto the pharynx. Last hearts in XII; those in X–XII latero-oesophageal. Supra-oesophageal vessel in IX (and further forward?) to XII or XIII. Subneural vessel absent. Nephridia stomate vesiculate holonephridia; postseptal bodies commencing in II; bladders with or without lateral diverticula. Testes and funnels free,

brown dorsally, paler ventrally; no paler intersegmental strips. First few segments single; thereafter with a tendency to a biannulate condition, with presetal furrow. Prostomium epilobous, open, $\frac{1}{3}$, $\frac{1}{2}$, with dorsal median groove which extends throughout the body. Peristomium not bisected ventrally. First dorsal pore 4/5 (rudimentary), 5/6 well developed. Setae commencing on II, in 8 regular long-

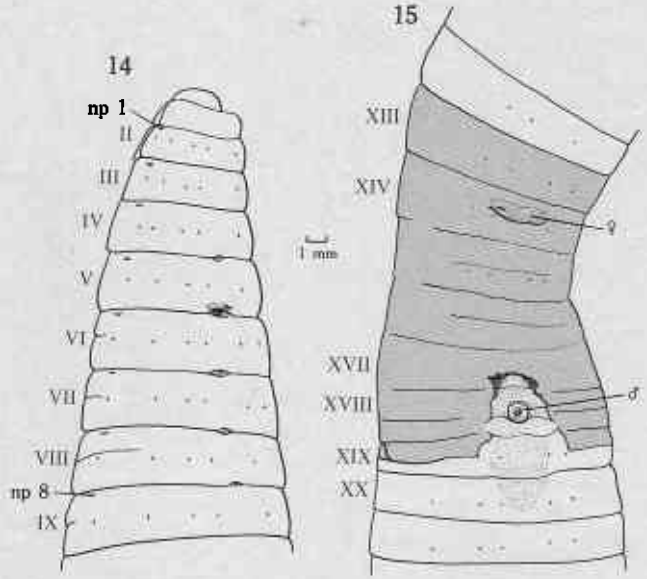


Figs 11–13. *Fletcherodrilus unicus* (Fletcher, 1889): *11*, genital field of specimen 1, Lamington, QM G8795; *12*, dorsal view of prostomium and anterior segments; *13*, genital field of specimen 10, Numinbah Valley, BJ 1978.5.34.

itudinal rows excepting caudally where all rows are irregular; *a* and *b* absent unilaterally, or bilaterally, in XVIII. In XII *aa* : *ab* : *bc* : *cd* : *dd* = 1·8 : 1·0 : 1·8 : 2·0 : 9·6 (mean of 8). Nephropores conspicuous slits at the anterior border of their segments, a pair in *d* lines in V posteriorly; those in II, III and IV progressively further dorsal anteriorly, those of II lying about halfway between *d* and the middorsum. Clitellum (S1; not developed in S2) annular, protuberant, well developed in XIV–XVII but some clitellar development apparently present from $\frac{1}{2}$XIII to XVIII; dorsal pores obscured but intersegmental furrows and setae retained. A single pore of the pair of prostates and the vasa deferentia midventral in XVIII,

a small circular orifice concealed in the equatorial but presetal furrow. Accessory genital markings absent. Female pores small, shortly anteromedian of setae *a* of XIV. Spermathecal pores unpaired midventral, concealed in the 5 intersegments 4/5–8/9.

Septa 9/10–15/16, 16/17 strongly thickened, with 13/14 and 14/15 the thickest. Dorsal blood vessel single, continuous onto the pharynx and passing under the brain. Last hearts in XII; those in X–XII large latero-oesophageal, each receiving a broad connective from the supra-oesophageal vessel and a very slender connective from the dorsal vessel; commissurals in VII–IX slender, dorsoventral only and differing from the hearts of X–XII in giving off branches to the septa and body wall shortly before joining the ventral vessel. Supra-oesophageal vessel traceable in ½X, X–XII, ½XIII. Subneural vessel absent. A pair of large latero-oesophageal vessels arising from the suboesophageal vessel in X and passing forward to the wall of the pharynx. A pair of latero-parietal vessels arising beneath the oseophagus in XIII and running posteriorly, crossing the prostate ducts.



Figs 14 and 15. *Fletcherodrilus fasciatus* (Fletcher, 1890), specimen 1, Binnaburra, BJ 1978.5.3; *14*, anterior extremity showing spermathecal pores and nephropores; *15*, male genital field.

A large, strongly muscular gizzard with the form of an anteriorly widening truncated cone or almost cylindrical, in VI, preceded in IV and V by narrower or equally wide tortuous oesophagus. The oesophagus in VII and VIII narrow, non-vascular, largely suppressed by backward extension of the gizzard; in IX–XV (S2) or XVII (S1) segmentally dilated; in XIII, XIV and XV expanded to form 3 pairs of broadly sessile lateral uniform calciferous glands, largest in XIV. These glands with numerous radial lamellar villi which are not, however, high and do not unite, the lumen of the glands not separated from that of the oesophagus. Intestine commencing at the anterior limit of XVIII, wide and chloragogenous; muscular thickening, caeca and typhlosole absent.

Nephridia stomate vesiculate holonephridia, commencing in II; each with a preseptal funnel; the funnels very large in the intestinal region. Nephridial bladders large, at first elongate sacs but by XI or X with knob-like rudiment of a diverticulum which by XI (S2) or XIII (S1) has become almost as large as the ental region of the bladder. By the intestinal region the bladders are transversely elongate and the diverticulum, of equal width, extending laterally from the pore is almost $\frac{1}{4}$ the length of the portion median to the pore. Entry of the nephridial ducts into the bladders is slightly penultimate. Free testes and iridescent fairly small sperm funnels in X and XI (S1; funnels not detectable in S2) multiloculate, iridescent, racemose seminal vesicles in XI and XII, those in XII slightly the larger. Weblike ovaries with many conjoined strings of large oocytes, and funnels in XIII; ovisacs absent. Prostates restricted to XVIII in which the thickly tubular glandular portion passes laterally from the duct. The duct consisting of an ental straight slender portion and ectal thick, initially once coiled, strongly muscular portion which joins that of the other side within a muscular mound, midventral on the body wall, beneath the ventral nerve cord. Vas deferens joining the thick portion of the muscular prostate duct immediately ectal of the coil. Spermathecae 5, unpaired, discharging anteriorly in V–IX; each with an ovoid to sacciform ampulla (on one side or the other of the nerve cord) and a well demarcated, thinner, though short, muscular duct which is joined at the body wall by 2 digitiform to slightly clavate inseminated diverticula; size approximately uniform; length of spermatheca of IX, 1·9 mm; ratio of total length : length duct, 3·1; ratio length : length diverticulum, 1·9 (S1).

Material Examined

Queensland: 28°19′S., 153°05′E. (approx.), Lamington National Park, in montane rain forest, *c.* 2 km from O'Reilly's Guest House, in a rotting log, J. E. Wampler and B. G. M. Jamieson, 14.i.1978, specimens 1 and 2 (QM G8795, 8796). 26°23′S., 153°07′E., Noosa, in rotting log in rain forest on sand, J. Wampler and G. R. Dyne, 1.ii.1978, specimens 3, 6–8 (QM G8833, BMNH 1979.1.2, BJ 1978.5.1,2). 26°51′S., 151°34′E., Bunya Mountains National Park, J. E. Wampler and G. R. Dyne, 1.ii.1978, specimen 4 (QM G8847). 24°56′S., 145°32′E., Ravensbourne National Park, under leaf litter and rotten logs in rain forest during rain, J. E. Wampler and G. R. Dyne, 1.ii.1978, specimens 5 and 9 (BMNH 1979.1.1, BJ 1978.5.34). 28°08′S., 153°14′E., Numinbah Valley, in soil near house, C. McKavanagh, 20.viii.1966, specimen 10 (BJ 1975.4.2).

Other Records

New South Wales: (*1*) 30°20′S., 149°47′E., Narrabri, and 31°16′S., 149°17′E., Coonabarabran (Type-localities); (*2*) banks of Lake Cudgellico, a few miles from the Lachlan River. **Queensland:** (*3*) 25°37′S., 151°37′E., Gayndah; (*3*) 22°56′S., 148°05′E., Peak Down Station; (*4*) 24°20′S., 151°34′E., Miriam Vale; (*4*) 21°42′S., 150°20′E., Percy I.; (*5*) Christmas Creek; 26°34′S., 152°52′E., Blackall Range; 24°24′S., 151°37′E., Colosseum; Glen Lamington; (*6*) Cape York?. Michaelsen (1891) gave the locality of var. *pelewensis* as Pelew Islands but later (1900) stated that this was erroneous.

Luminescence of Fletcherodrilus unicus

F. unicus is weakly to moderately luminescent ($\sim 10^8$ photons s^{-1}) and requires full dark adaptation to be seen. Evidence for a particulate luminescence system was obtained by the same procedure as described for *S.* (*S.*) *curtisi*. The resuspended particles were stimulated to emit upon addition of distilled water and further stimulated (2–8×) by subsequent addition of hydrogen peroxide. Both extracts of whole fluid and separated particles were stimulated by peroxide addition

and strongly stimulated by the subsequent addition of *Diplocardia* luciferase (20–100×). *Diplocardia* luciferin, on the other hand, had no effect.

Similarly to that in *S.* (*S.*) *curtisi*, the spontaneous level of emission from *F. unicus* was quite variable and not detectable in some specimens. In each case, however, addition of peroxide followed by *Diplocardia* luciferase gave detectable emission.

Fletcherodrilus fasciatus (Fletcher)

(Figs 10, 14, 15, 19, 20; Table 6)

Cryptodrilus (?) fasciatus Fletcher, 1890, pp. 988–9 (*1*)*.
Fletcherodrilus unicus var. *fasciatus;* Michaelsen, 1891, p. 32.
Fletcherodrilus unicus fasciatus; Michaelsen, 1900.
Fletcherodrilus fasciatus; Jamieson, 1971*b*; p. 85 (*2*).

Description

This account refers to specimens 1 and 3 (S1 and S3) unless otherwise stated.

Table 6. Intersetal distances in *Fletcherodrilus fasciatus*
Values are for specimens 3 and 4

	Percentage of circumference					Circumference
	aa	*ab*	*bc*	*cd*	*dd*	(mm)
Segment XII						
Specimen 3	8·12	3·74	7·91	8·80	50·98	18·97
Specimen 4	8·27	4·48	7·09	10·57	47·48	17·65
Minimum	8·12	3·53	6·52	8·80	47·48	
Maximum	8·27	4·59	7·91	11·10	50·98	
Mean (a,b)	8·20	4·11	7·50	9·68	49·23	
Mean, interval/*ab*	2·01	1·00	1·85	2·36	12·12	
Segment XX						
Mean, interval/*ab*	2·46	1·00	2·08	2·69	11·39	

Length 128, 290 mm, width (midclitellar) 7·0, 7·3 mm, segments 98, 127. Dorsally and laterally encircled by deep purple-brown segmental strips separated by unpigmented intersegmental strips. First few segments single; thereafter faintly biannulate, with presetal furrow. Ventrally pale with very slight indication of the dorsal striping. Clitellum (S1) unstriped, uniform chocolate brown. Cross-section appreciably depressed dorsoventrally. Prostomium wide, slightly epilobous. Peristomium not bisected ventrally, though with some fine longitudinal grooves. First dorsal pore 3/4 (rudimentary), 4/5 perforate. A narrow dorsal groove present throughout, well developed in the first few segments, faint further posteriorly. Setae commencing in II, in 8 regular longitudinal rows throughout; *a* and *b* absent in XVIII. In XII $aa : ab : bc : cd : dd = 2{\cdot}0 : 1{\cdot}0 : 1{\cdot}9 : 2{\cdot}4 : 11{\cdot}8$ (mean of 3). Nephropores conspicuous slits near the anterior borders of their segments, in II to the posterior end, all in *d* lines. Clitellum (S1) annular, tumid but constricted; XIII–$\frac{1}{2}$XIX, interrupted in $\frac{1}{2}$XVII–XIX (maximally in XIX) throughout *bb* by the

* Numerals refer to 'Material Examined' and 'Other Records', p. 663.

whitish glandular field which includes the median male porophore; dorsal pores obscured, intersegmental furrows and setae less clear than elsewhere, nephropores retained. A single pore of the pair of prostates and the vasa deferentia midventral in XVIII on a hemispheroidal strongly protuberant equatorial male porophore which bears the male pore on a small truncated cone (S1) or as a transverse slit (S3), the two conditions presumably representing eversion and retraction of the terminal ducts; the base of the porophore filling approximately $\frac{1}{3}$ *aa*. A diamond-shaped midventral whitish glandular field in $\frac{1}{2}$XVII–$\frac{1}{2}$XX, filling *aa* at its greatest width, in XIX, deeply insunk in XVII and anterior XVIII and interrupting the clitellum from $\frac{1}{4}$XVII posteriorly (S1); faintly indicated in S3. Accessory genital

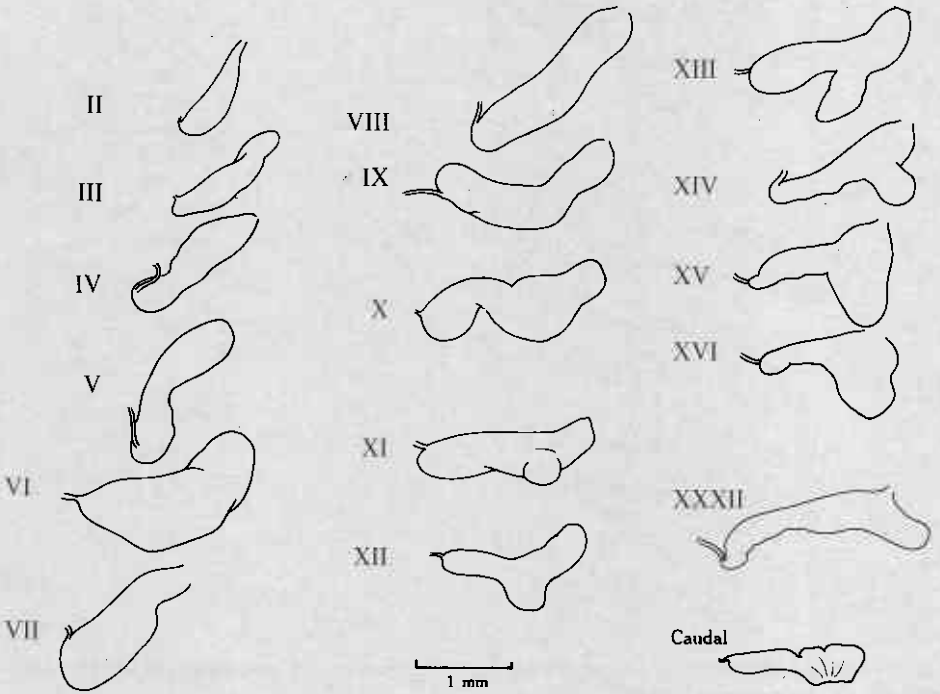
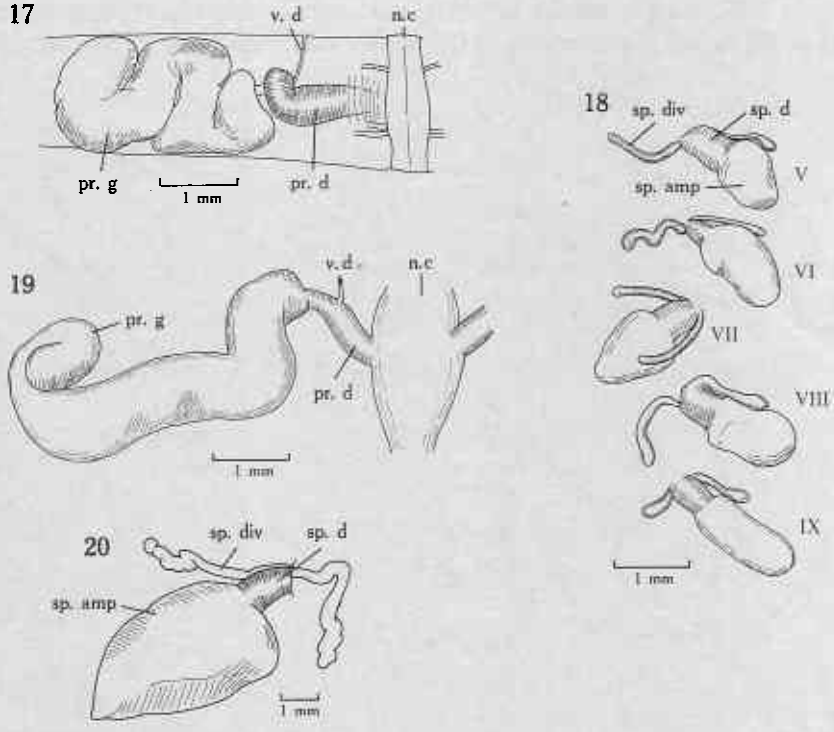


Fig. 16. *Fletcherodrilus unicus* (Fletcher, 1889), specimen 1, Lamington, QM G8795. Nephridial bladders of right side in segments indicated.

markings absent. Female pores small paired transverse slits, shortly anteromedian of setae *a* of XIV (S1, S3); on development of the clitellum seen to lie in a common whitish tumid field (S1). Spermathecal pores unpaired midventral, small but with considerable elliptical borders, in intersegments 4/5–8/9.

Septa 4/5–16/17 thickened; 4/5–8/9 only slightly to moderately, 9/10–12/13 increasingly strongly with 12/13 very strong; 13/14–16/17 less thickened but still strong; 4/5–8/9 (S3)–10/11 (S1) funnel-shaped (dependent on state of contraction?). Dorsal blood vessel single, continuous onto the pharynx. Last hearts in XII; those in X–XII latero-oesophageal, slender in X, very large in XI and XII, each receiving a connective from the dorsal vessel and one from the supra-oesophageal vessel. Commissurals in VII–IX dorsoventral only, differing from the hearts of X–XII in giving off lateral, parietal branches. Supra-oesophageal vessel

slender, traceable in IX–XII (S1), ½XIII (S3), but probably continuous with a very slender vessel in VII (S1), not present behind the anterior region of the first calciferous glands (XIII and XIV) but presumably confluent with their sinuses; apparently fusing with the dorsal vessel at septum 9/10. Subneural vessel absent. A pair of transverse parietal vessels present in each oesophageal segment; the vessels in XIV continuous via a longitudinal lateroparietal with those of posterior segments at least as far as XIX.



Figs 17 and 18. *Fletcherodrilus unicus* (Fletcher, 1889), specimen 1, Lamington, QM G8795: *17*, left prostate; *18*, spermathecae in situ.

Figs 19 and 20. *Fletcherodrilus fasciatus* (Fletcher, 1890), specimen 1, Binnaburra, BJ 1978.5.3: *19*, left prostate; *20*, spermatheca of IX.

A large cylindrical gizzard in VII, ensheathed in the diaphanous septum 7/8; which in S3 is so firmly adherent to the anterior portion of the gizzard that most of the gizzard appears to lie in VIII; backwardly displaced so that its hind end lies at 9/10 relative to external segmentation. Postgizzard oesophagus beginning in IX. Oesophagus in XIII and XIV forming large, lateral pouch-like swellings which are in no way constricted off or separate from the central oesophageal lumen; those of the 2 sides separated by a deep groove (S3) or united medianly so as to form an annular (calciferous?) gland in each segment. Each gland has low lamellar internal folds which contain blood sinuses and externally longitudinal striations corresponding with these lamellae. Intestine commencing at the anterior limit of XVIII; muscular thickening, caeca and typhlosole absent. Nephridia stomate, vesiculate holonephridia, commencing in II (preseptal funnel demon-

strated for those of V, S1). First 3 pairs with long club-shaped bladders which the end tube enters immediately subterminally (segment II) or distinctly subterminally (segment IV). From the 4th pair posteriorly each bladder has a well developed lateral caecum, as large as or larger than the bladder. Free testes and iridescent sperm funnels in X and XI. Seminal vesicles small, smooth surfaced, in IX and X; large, with some deep incisions, in XI and XII. Weblike ovaries with many conjoined strings of oocytes, and funnels, in XIII; ovisacs absent. Prostates restricted to XVIII in which the thickly tubular tortuous glandular portion passes laterally from the duct (S1) or is wound on itself in a single plane and greatly depressed (S3). The duct almost straight, widening to join a median mound corresponding with the porophore. Vas deferens joining the prostate duct at approximately its midlength. Spermathecae 5, unpaired, discharging anteriorly in V–IX, each with an ovoid ampulla (on one side or the other of the nerve cord) and a well demarcated, slender, though short, muscular duct which is joined at the body wall by 2 slender almost tubular, slightly clavate, beaded, inseminated diverticula; size of spermathecae increasing posteriorly (S1, 3), length of spermatheca of VII (S1) = 6·7 mm, ratio of total length : length duct = 5·3; ratio length: length diverticulum = 1·3.

Material Examined

Queensland: (*2*) 28°17′S., 153°11′E., Binnaburra, Lamington National Park, in rain forest soil, G. Grigg, 25.v.1961, specimen 1 (BJ 1978.5.3.); 28°14′S., 153°08′E. (approx.), near O'Reilly's Guest House, in a rotting log in rain forest, J. E. Wampler and B. G. M. Jamieson, 11.i.1978, specimen 2 (BJ 1978.5.4), 14.i.1978, specimens 3 and 4 (QM G8916, 8917).

Other Records

New South Wales: (*1*) 28°25′–29°04′S., 152°46′–153°21′E. (approx.), Richmond River District (Type locality).

Luminescence of F. fasciatus

F. fasciatus was very weakly luminescent ($\sim 10^7$ protons s^{-1}) requiring full dark adaptation to be seen. Emission was only moderately stimulated by peroxide addition (~2×) and somewhat more stimulated by subsequent addition of *Diplocardia* luciferase (2–4×). No evidence for particulate luminescence was obtained.

Genus **Pontodrilus** Perrier

Definition

See Jamieson (1971*b*, p. 89).

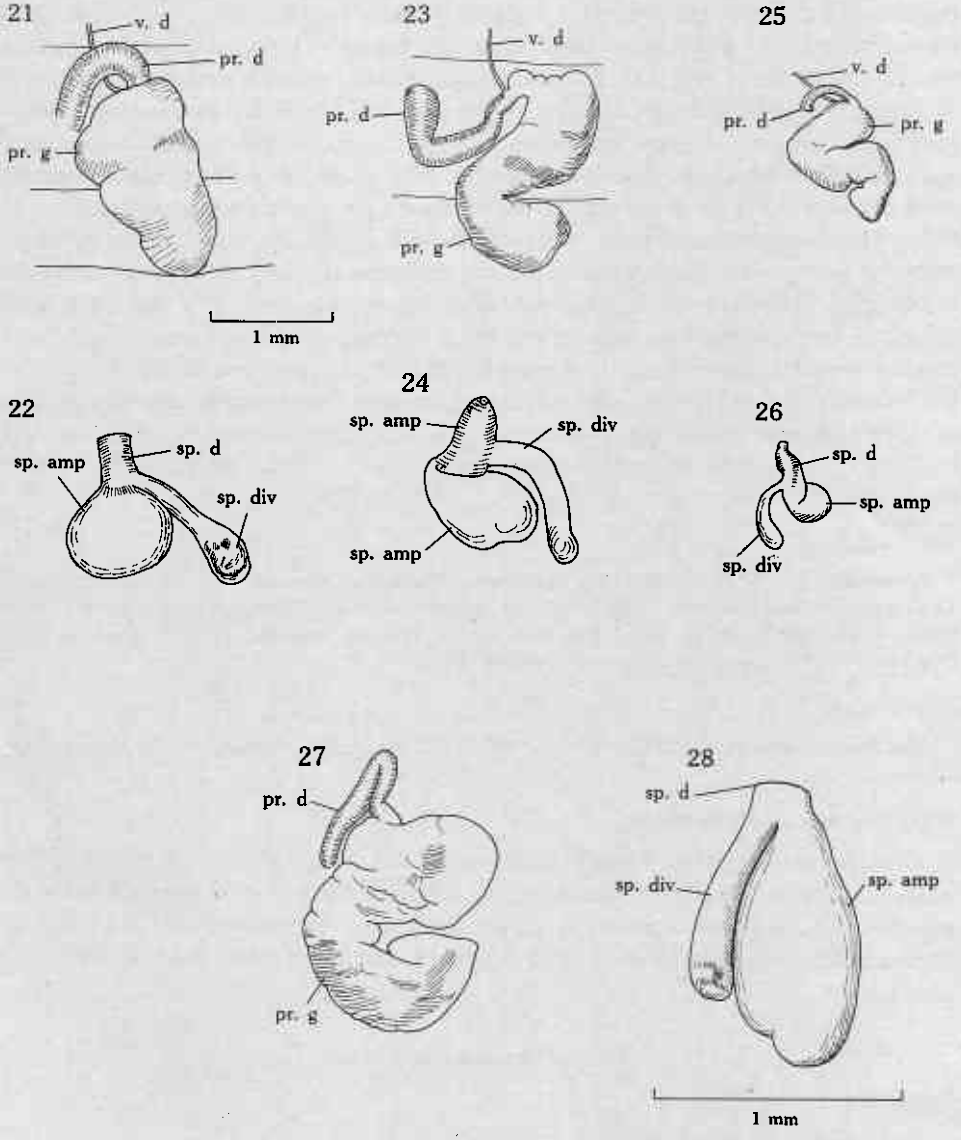
Distribution

Circummundane in the marine to brackish littoral in the tropics and warmer temperate regions.

Type-species: *Lumbricus litoralis* Grube, 1855 (southern France).

Four species, with numerous junior synonyms, and two terrestrial and lacustrine species doubtfully attributable.

Two species are known from Australia: *Pontodrilus litoralis* (Grube, 1855) and *P. bermudensis* Beddard, 1891. Only the latter is available for bioluminescence studies.



Figs 21 and 22. *Spenceriella* (*S.*) *cormieri*, sp. nov., holotype QM G8918: *21*, right prostate; *22*, right spermatheca of IX.

Figs 23 and 24. *Spenceriella* (*S.*) *curtisi*, sp. nov., holotype QM G8897: *23*, right prostate. *24*, left spermatheca of IX.

Figs 25 and 26. *Spenceriella* (*S.*) *minor* (Spencer, 1900), specimen 1, Tamborine: *25*, right prostate; *26*, right spermatheca of IX.

Figs 27 and 28. *Spenceriella* (*S.*) *noctiluca*, sp. nov., holotype QM G8922: *27*, right prostate; *28*, left spermatheca of IX.

Figs 21–27 are to same scale.

Pontodrilus bermudensis Beddard

Major synonyms and Australian references:

Pontoscolex arenicola Schmarda, 1861 (part.), p. 11.
Pontodrilus bermudensis Beddard, 1891, pp. 88–96; Stephenson, 1931, p. 51; Rao, 1974, pp. 1–173.
Cryptodrilus insularis Rosa, 1891, pp. 387–8.
Pontodrilus arenae Michaelsen, 1892, pp. 209–61.
Pontodrilus hesperidum Beddard, 1894, pp. 37–40.
Pontodrilus michaelseni Eisen, 1895, pp. 73–84.
Pontodrilus ephippiger Rosa, 1898, pp. 227–90.
Pontodrilus ephippiger var. *laysanianus* Michaelsen, 1899, p. 217.
Pontodrilus bermudensis f. *ephippiger:* Jackson, 1931, pp. 71–136.
Pontodrilus michaelseni var. *hortensis* Eisen, 1900, p. 241–3.
Pontodrilus laccadivensis Beddard, 1903, pp. 374–5.

Material Examined

Queensland: 27°31'S., 153°21'E., Peel I., Moreton Bay, in sand under, and between the leaves of, dead *Zostera* at approx. mean high tide level, Mr O. Kelly, 11.i.1978, 7 specimens (QM G8931), 7 specimens (BJ 1978.5.35).

Other Australian Records

Great Barrier Reef Expedition, General Survey (Stephenson 1931), south-west Australia (Jackson 1931), Low Isles, Great Barrier Reef (Stephenson *et al.* 1958).

Wider Distribution

Bermuda, Mexico, Bahamas, Dry Tortugas, Haiti, Jamaica, Virgin Is, Mona I. Colombia, Brazil. Congo, Angola, South Africa, Tanzania, Madagascar. Laccadive Is, India (east and west coasts). Maldive Is, Ceylon. Palmyra Atoll, Fannin I., Laysan. Texas, Mississippi, Louisiana, Florida, Virginia. Cape Verde Is. Burma, Andaman Is. Car Nicobar. Vietnam, Hainan, Java, Christmas I., Borneo, Celebes, Aru, New Guinea. Loyalty Is. Tonga.

Remarks

The new specimens have spermathecal and male pores in *b* lines. The male pores are flanked by high longitudinal ridges and there is a transverse trough with raised margins in 19/20. No other accessory genital markings are present. These external genital features characterize *P. bermudensis*, with its junior synonyms, and *P. matsushimensis*. The Peel I. specimens are identified as *P. bermudensis* by the muscular prostate ducts, which are absent in *P. matsushimensis*. The latter species, occurring in Japan and New Zealand, is possibly conspecific with *P. bermudensis*. Similar reduction of the prostate ducts is ascribed to parthenogenetic degradation in, for instance, *Pheretima* species.

Luminescence of Pontodrilus bermudensis

P. bermudensis is brightly luminous, being easily visible to the non-dark-adapted eye. While extensive testing was carried out, no evidence for cell-bound luminescence was obtained. When experiments analogous to those described for *S.* (*S.*) *noctiluca* were performed, the separated cells were seen to be smaller and their contents less homogeneous than in *D. longa* or *S.* (*S*). *noctiluca*. They contained little bioluminescence activity.

The soluble extract was slightly stimulated by addition of dilute hydrogen per-

oxide and strongly stimulated (6–10×) by subsequent addition of *D. longa* luciferase. Slight stimulation (3×) by *Diplocardia* luciferin was also observed.

Summary of General Observations of Luminescence

In all these earthworm species, the bioluminescence originated from exuded fluid following electrical or tactile stimulation, or both. In no case was the body of the earthworm bioluminescent. Centrifugation of a suspension of fluid in phosphate buffer resulted in separation of active particulate material from *S. curtisi, S. noctiluca* and *F. unicus*. Together with the observation of chloragogen-like coelomocytes from *S. noctiluca*, this suggests that the luminescence is packaged in a similar way in these species. In *Pontodrilus bermudensis*, however, no particulate luminescence system could be demonstrated. Lynch (personal communication) has previously reported that the bioluminescence of *Pontodrilus matsushimensis* is not cell-bound.

Table 7. Summary of comparative physiological and biochemical data

Stimulation indicated by +; no stimulation relative to control experiments indicated by −; variable results. ±; item not tested or examined. 0. Strong stimulation indicated by addition + sign per order of magnitude

Species	Luminescent exudate	Bioluminescence		Peroxide stimulation	Crude cross-reactions with:	
		Peak light (photons s^{-1})	Particulate source		*D. longa* luciferase	*D. longa* luciferin
S. (S.) cormieri	Yes	10^{11}	–	+	±	+
S. (S.) curtisi	Yes	$10^{8}-10^{9}$	Yes	+	++	–
S. (S.) minor	Yes	$10^{9}-10^{10}$	0	+	+	+
S. (S.) noctiluca	Yes	10^{9}	Yes	+++	+	±
F. unicus	Yes	10^{8}	Yes	+	++	–
F. fasciatus	Yes	10^{7}	0	+	±	±
P. bermudensis	Yes	10^{9}	No	+	+	+
D. longa	Yes	$10^{10}-10^{11}$	Yes	++	++	++

As pointed out in the individual sections above, all *in vitro* reactions were stimulated by addition of dilute hydrogen peroxide. In all cases either *Diplocardia* luciferase of *Diplocardia* luciferin stimulated the *in vitro* reactions following addition of peroxide. Table 7 summarizes the data on luminescence of these earthworms compared with those for *Diplocardia longa*.

Both the levels of luminescence and the stimulating effects of additions to the *in vitro* reactions were variable from specimen to specimen within a given species. Jamieson (1977*a*) had previously found a non-luminous specimen of *Digaster keasti* which exhibited bioluminescence when dilute hydrogen peroxide was added to the exuded coelomic fluid. His data, along with data reported here for *S. curtisi* and *F. unicus*, suggest that the capacity for luminescence is determined by the availability of peroxide, or of the enzyme luciferase, or of both. Their availability could, in turn, be controlled by environmental, nutritional or developmental factors. Surprisingly, there seems to be no lack of the substrate, luciferin, in any of the specimens. Luciferin activity has also recently been found in several non-luminous species which are related at the genus level to luminescent ones (Wampler and Jamieson, unpublished). On the other hand, species of *Heteroporodrilus* show no

trace of luciferin or luciferase cross-reactivity with the *Diplocardia* system, and no peroxide stimulation.

These observations of variable capacity to luminesce raise questions about the role of bioluminescence in earthworms. Many of the species reported here and observed by others create a brilliant display. It seems unlikely, therefore, that light emission is a trivial by-product of the animal's chemistry. Generation of light requires a great deal of energy which must be released during concerted chemical events. An electronic excited state must be formed and, in addition, the excited emitter must not be quenched by adjacent molecules before light is emitted. Fortuitous emission accompanying some metabolic process is certainly possible, but efficient emission from this cause seems unlikely, as does retention of the efficiency of luminescence throughout evolution of species. Another argument against this trivial role of bioluminescence in earthworms is the nature of the luminescence event. Since worms exude a bioluminescence system which is subsequently activated, a role for the chemistry itself seems unlikely. Metabolic changes in the coelomic fluid at this stage should not be necessary, as the worm has already separated itself from the fluid and its contents.

The best argument for a functional role for the luminescence is that earthworms do, after all, live in a dark environment. Thus, like deep-sea creatures, they *could* utilize low-level emission to advantage. With marine organisms, bioluminescence serves many varied functions: light lures for predation, defensive displays, light aids to vision, countershading, and social purposes (Morin *et al.* 1975; P. J. Herring 1977). Since earthworms are not predatory and do not have a developed visual system (they can detect and respond to light, however; see Mill 1978), a defensive or social role seems most likely. The observation of luminescence in earthworms has been constantly associated with adverse stimulation (Atkinson 1887; Lloyd-Bozward 1897; Benham 1899; McDermott and Barber 1914; Gates 1925, 1944; Skowron 1928; Pickford 1937; Johnson *et al.* 1965), therefore defence is the most obvious hypothesis.

There are strong arguments against this hypothesis, however. Many workers note difficulty in stimulating the luminescence reaction. Several species, for example *Octochaetus*, respond to the initial stimulation by becoming very rigid and immobile, and only after considerable rough treatment or injury do they exude the slime. In some species, the variability of luminescence noted above also argues against a defensive role for bioluminescence. Other explanations must be examined and, as with marine creatures, the possibility that the role of luminescence may vary from species to species must be considered.

Luminescence could be a warning to predators of an unpalatable or toxic species, or in some it could serve a more complex sexual or social function. Support for these hypotheses can only be obtained by examining the behaviour and life cycles of different species of bioluminescent earthworms.

Despite the possibility of varying biological functions, the data of Table 7 suggest strong homology between the biochemical and physiological systems of bioluminescence in these Australian species and in *D. longa* from North America. Further comparisons between North American and Australian earthworms and studies of the chemical and biological systems of their bioluminescence will continue under the Cooperative Science Project between these laboratories.

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