Proc. R. Soc. Qd **88:** 83–88 (1977). ISSN 0080-469X

Bioluminescent Australian Earthworms, I. *Digaster keasti* sp. nov., (Megascolecidae), the first record of an Oligochaete from Fraser Island

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Received 30th November 1975

ABSTRACT

Digaster keasti sp. nov. is described and is compared with the closely related *D. longmani* and *D. brunneus*. It occurs on Fraser Island and on the adjacent mainland, in South-east Queensland, between the ranges of the other two species. *D. keasti* exhibits moderate luminescence which appears to be of the peroxidative type. The biological significance of the luminescence is unknown. The evolution and significance of bioluminescence are briefly discussed. The evidence contraindicates a defensive function in *D. keasti*. Specimens of *D. brunneus* are shown to lack luminescence.

INTRODUCTION

Biqluminescence has been reported in thirteen genera of oligochaetes belonging to three families: *Enchytraeus*(?), *Michaelseniella*(?) and *Henlea* Enchytraeidae), *Eisenia* (Lumbricidae) and nine genera of the Megascolecidae which are classifiable as follows in the revised classification of Jamieson (1971): *Diplocardia*, *Microscolex* (including '*Eodrilus*' species) and *Parachilota* (Acanthodrilinae, tribe Acanthodrilini); *Octochaetus* (Acanthodrilinae, tribe Octochaetini); *Pontodrilus* (Megascolecinae, tribe Perionychini), *Eutyphoeus* and *Ramiella* (Megascolecinae, tribe Dichogastrini); and *Lampito* (Megascolecinae, tribe Megascolecini) (Harvey, *fide* Pickford, 1952; Bellisario, Spencer and Cormier, 1972).

To this list can now be added the dichogastrin genus *Digaster* in which *Digaster keasti* is here shown to be luminescent as a result of investigation of a live specimen from Fraser Island kindly donated to the author by Professor Allen Keast. This constitutes the first record of an oligochaete from Fraser Island and the first report of luminescence in an Australian oligochaete. Mr R. Raven has drawn the author's attention to further material of this species from the island and adjacent mainland in the collections of the Queensland Museum. This account will chiefly be limited to a taxonomic description of the new species, but a preliminary investigation of its luminescence is also reported.

SYSTEMATICS

Digaster keasti sp. nov.

(Fig. 1 Table1)

The following description is taken from the holotype, H, from Fraser Island (QM G8482), and paratype 3, P3, from the Toolana State Forest (QM G8869) respectively. Brief reference to the other 4 specimens is also included.

1 = 416 710 mm; midclitellar width = 12, 18 mm; greatest width (forebody) = 14–21 mm, s = 275 379. Body in life weakly pigmented greyish brown, pale ventrally, clitellum deep purplish brown (H). Circular (H) or anteriorly dorsoventrally depressed (P3) in cross section. Secondary annulation so pronounced as to obscure basic segmentation, elucidation of which is further retarded by absence of visible preclitellar setae and of dorsal pores; segments V–XIII quadriannulate; the preceding segments

biannulate but with one (the peristomium?) uniannulate (simple); clitellar and succeeding segments distinctly biannulate, the two annuli equal, with the setae on the posterior annulus at or anterior to its equator; by XXVII the intra-segmental groove almost indistinguishable and succeeding segments simple. Prostomium broad, short, prolobous, it and the first one or two segments with numerous longitudinal grooves. First dorsal pore 5/6 in all specimens excepting the holotype in which it is not certainly visible until 20/21. Setae 4 pairs per segment, minute, the rows very irregular caudally though the ventral couples each continue to form a recognizable pair; irregularity developing shortly behind the clitellum, first in *d*, later in *c* and only posteriorly in *b* and then *a*. Nephropores not certainly recognizable. Clitellum well developed, annular, on XIV–XIX (H), XX (P3) as a prominent structure but at the dorsal incision epidermal thickening and pigmentation is demonstrable to ½ XXI and some pigmentation without thickening occurs through several more posterior segments; intersegments and secondary furrows distinctly visible or obliterated; setae distinct or faint (H, P3 respectively). Male pores short narrow transverse slits in *ab*, each on a flat-topped shortly cylindrical medianly inclined papilla which extends longitudinally from the posterior region of the anterior annulus to the hind margin of the posterior of the two annuli of XVIII; each papilla bounded laterally by a very prominent, whitish (non-clitellar) kidney-shaped lobe; the posterior part of the lobe extending into XIX and deflecting the equatorial groove of this posteriorly; the surface of the lobe in XIX bearing a large glandular patch. A pair of glandular strips (tubercula pubertatis) of similar texture to these patches present in posterior XIX to posterior XXII median to *b*, a raised whitish median strip intervening between them; the two longitudinal glandular strips tapering posteriorly so that the area enclosing them forms an elongate whitish triangle extending to 23/24 behind the male porophores (H).

Male porophores in all other specimens only weakly protuberant and reniform extension of the whitish non-clitellar field lateral to these either protuberant (QM 8871, 8872) as in the holotype or only slightly tumid (QM G8482, 8869, 8873). Posteriorly convergent tubercular pubertatis present behind the male porophores in all specimens. A pair of indistinct pads present in P3 posteriorly in XVII, at the anterior limit of the whitish field, median to *a* lines. Male pores 3.5, 7.0 mm, 0.10, 0.13 body circumference apart (H, P3). Female pores a minute pair, very close together shortly anterior to and far median of setae *a* of XIV (H, QM G8870). Spermathecal pores 2 pairs at the anterior margins of VIII and IX, each a conspicuous short transverse slit with narrow anterior and posterior lips forming an ellipse, in *ab* so far as the extremely vestigial and usually undemonstrable setae of the forebody allow determination; the posterior pair 5.3, 8.8 mm, 0.12, 0.14 body circumference apart (H, P3).

Thickest septa 5/6—12/13, very strongly thickened. Dorsal blood vessel single, continuous onto the pharynx; dorsoventral commissural vessels in IX, and anteriorly, branching to give off septal vessels shortly before joining the ventral vessel; those in X–XII unbranched ventrally but forming latero-oesophageal hearts, each receiving a connective from the supra-oesophageal vessel and from the dorsal vessel; supra-oesophageal vessel in ½IX–½XIII. Sub-neural vessel absent. Oesophagus in V enlarged and globose, gizzard-like but thin walled; a very large globose strongly muscular gizzard in each of VI and VII, a length of thin walled, unmodified oesophagus approximately half as long as a gizzard intervening between them.

Oesophagus with no obvious vascularization, dilatation or calciferous glands; narrow in VIII–XIV and imperceptibly merging with the intestine, the origin of which is therefore not certainly determinable though appearing to be XVIII in P3. Intestine conspicuously expanded in XIX posteriorly; muscular thickening, caeca and typhlosole absent. Very numerous closely spaced minute (not evidently tubular) astomate exonephric parietal micromeronephridia visible in V posteriorly, becoming arranged in transverse bands in approximately XVIII posteriorly; large racemose bodies of glandular appearance anteriorly in III and IV do not appear to be tufted nephridia and are apparently septal glands, those in III attaching medianly to the pharynx. (H, P3). Caudally with a large exonephric megameronephridium, with preseptal funnel median to sparse micromeronephridia (H).

Small non-iridescent sperm funnels in X and XI; very large much lobulated seminal vesicles in XII (incipient metandry). Prostates racemose, broad depressed tongue-shaped glands each with a very short weakly muscular external duct largely concealed in the loose longitudinal musculature of the body wall (H, P3). Ovaries with several (H) of many (P3) long egg strings; funnels small (P3) or not recognizable (H) in XIII. Ovisacs absent(?) Spermathecae 2 pairs, in VIII and IX, each clavate or elongate ovoid tapering ectally but with no recognizably demarcated duct (H) or with a short, narrower though poorly demarcated duct (P3); joined dorsolaterally near its ectal extremity by a sessile spherical internally multiloculate inseminated diverticulum; an iridescent knob dorsolaterally on the duct (P3), with intramural sperm chambers but not forming a definite extramural diverticulum. Length left spermatheca of IX = 9.5, 6.2 mm (H, P3); ratio length: length diverticulum = 7.3 (H).

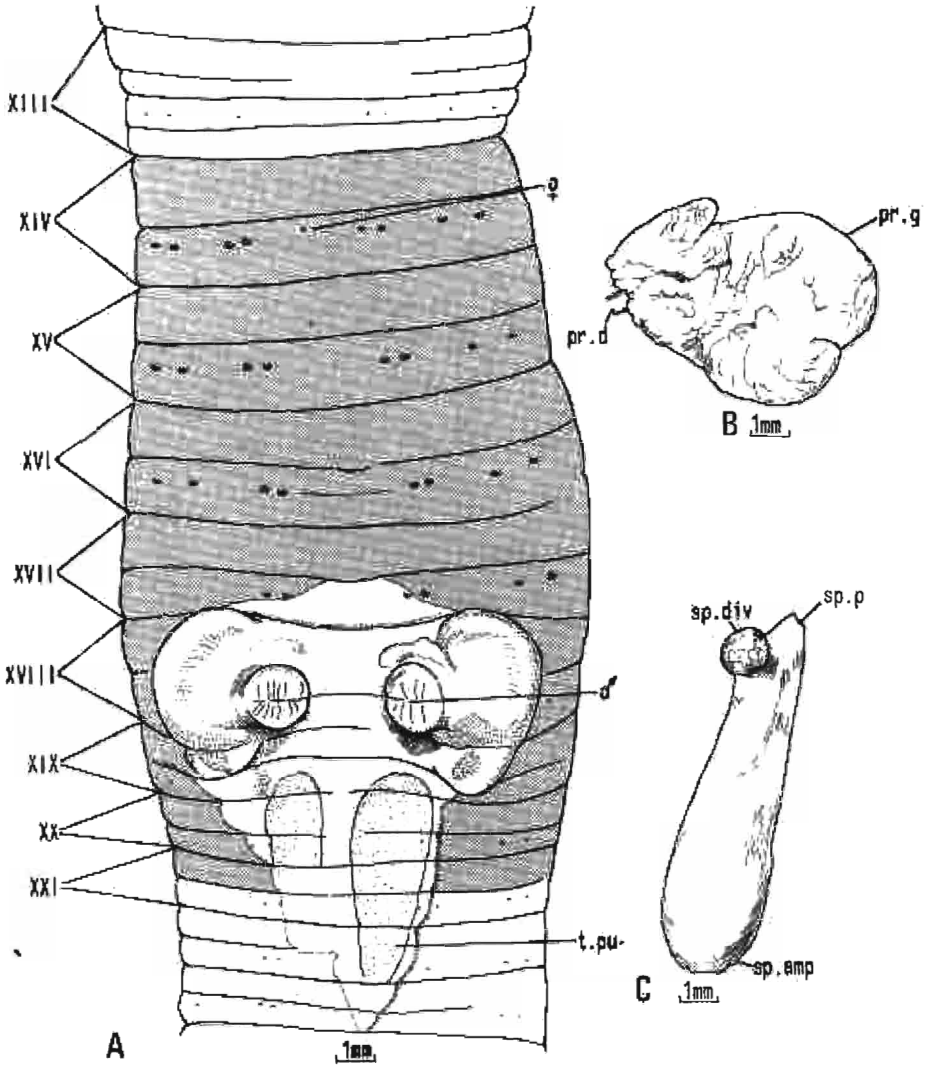

Fig. 1. *Digaster keasti* sp. nov., holotype. A, ventral view in the clitellar region. B, right prostate. C, left spermatheca. ♀, female pore; ♂, male pore; pr.d, prostate duct; pr.g, glandular portion of prostate; sp.amp., spermathecal ampulla; sp. div., spermathecal diverticulum, sp.p, spermathecal pore; t.pu. tuberculum pubertatis. Clitellum shaded. Roman numerals are segment numbers. By camera lucida.

Material Examined

153° 10′E, 25° 10′S, Fraser Island: Professor A. Keast, 16 May 1975, holotype, QM G8482; Australian National University collection, Jan. 1972, paratype 1, QM G8872. 152′ 40′E, 25° 16′S, Hervey Bay, heavy black loam, G. Dyne, 5 May 1974, paratype 2, QM G8873. 153° 02′E, 26° 10′S, Toolara State Forest: clearing in open forest, 25–30 cm below soil surface, J. James, K. McDonald, D. Crossman, 1 April 1974, paratype 3, QM G8869; sandy moderately damp soil in open forest, J. J. Kramadge, 11 April 1974, paratype 4, QM G8870. 153° 02′E, 26° 12′S, Locality R451 Cooloola, Forestry area, rainforest, approx. 8 km from Rainbow Beach entrance, sand with high humic content, K. McDonald, June 1972, paratype 5, QM G8871.

Remarks

The chief features in which *D. keasti* differs from the neighbouring *D. brunneus* are as follows. *D. keasti* is larger though *D. brunneus* overlaps the lower end of its range in length; the first dorsal pore is in

5/6 (not 9/10–11/12); transverse intersegmental ventral genital markings are absent and tubercula pubertatis are developed behind the male porophores; last hearts are in XII (not XIII); seminal funnels are in X and XI, the holandric condition, though an approach to the metandry of *D. brunneus* is seen in reduction of seminal vesicles to a pair in XII: and tufted nephridia are absent (not present in II–IV), the racemose structures in anterior segments in *D. keasti* being interpreted as non-nephridial. Furthermore, the setal ratios differ, notably *bc* which is significantly larger in *D. brunneus* relative to the circumference of the body (Table 1).

TABLE I: Intersetal distances in segment XX in *Digaster* as % of circumference

	aa	ab	bc	cd	dd	dc	cb	ba	mm circumference
D. keasti, holotype	14.1	1.7	5.6	2.5	65.6	2.5	6.5	1.4	27.6
QMG8870	12.0	1.1	9.3	5.5	57.1	3.5	10.5	1.0	32.0
D. brunneus, QMG8483	10.8	1.5	15.7	3.3	48.0	3.3	16.0	1.5	24.5
D. longmani, BJ1975.12.1	10.1	1.7	8.4	4.3	61.9	3.4	8.4	2.0	45.0

Some morphological intermediates between *D. brunneus* and *D. keasti* are nevertheless known on the mainland. Thus a specimen from Wolvi tentatively identified as *D. brunneus* by Jamieson (1975) agrees with the holotype of *D. keasti* in having genital lobes lateral to the male porophores, lacking transverse markings, and in location of the last hearts in XII but, like *D. brunneus*, is metandric and has the first dorsal pore in the vicinity of 9/10–10/11. Furthermore, specimens from Bauple (*loc. cit.*) which also have the last hearts in XII combine transverse pads, as in the typical population of *D. brunneus*, with an approach to the male genital lobes of the holotype, Wolvi and other specimens. Only the specimens assigned to *D. keasti* have the first dorsal pore in 5/6, tubercula pubertatis and two pairs of sperm funnels, however, and it is here considered that they warrant separate specific status. Whether the intermediate forms are themselves reproductively isolated from *brunneus* and *keasti*, though of presumed common ancestry, or indicate introgression of the two taxa awaits further investigation. It will be shown below that *D. keasti* differs further from newly obtained live specimens of *D. brunneus* in being bioluminescent.

Relationship of *D. keasti* with the similarly very large species *D. longmani* from Mt Tamborine, shortly to the south, appears to be close. The latter species agrees in having the first dorsal pore in 5/6 and has closely similar setal ratios (Table 1). It differs notably from *D. keasti* in absence of sperm funnels from X and of tubercula pubertatis and in typical location of last hearts in XIII, though these lie in XII in some forms. Tufted nephridia have not been reported.

Morphological resemblance between *D. keasti*, *D. brunneus* and *D. longmani* is sufficiently close to suggest closer phylogenetic relationship of the three species to each other than to any other species. Whether reproductive isolation is complete between them remains to be demonstrated.

LUMINESCENCE IN DIGASTER

It has been reported (Bellisario *et. al.*, 1972) for the North American Acanthodriline species *Diplocardia longa* that its luminescence belongs to a class of peroxidative bioluminescent reactions in which hydrogen peroxide (H_2O_2) is utilized for light emission instead of molecular oxygen. In that species luciferase and luciferin are located within coelomocytes and luminescence is produced in coelomic fluid, supposedly after lysis of contained coelomocytes, following exudation from the dorsal pores in response to mechanical or electrical stimulation. The luciferase is a non-haeme protein which is not itself a peroxidase. H_2O_2 acts on luciferin to give light plus products under the action of luciferase but the latter enzyme does not demonstrate normal enzymatic catalysis and 'turnover' for it is irreversibly inactivated by H_2O_2. It is believed that the H_2O_2 is generated in the presence of molecular oxygen by an oxidase contained in the coelomic fluid. Thus the H_2O_2 which is required for light emission, yet destroys the luciferase, is prevented from exerting these effects until the exuded coelomocytes undergo lysis. The biological significance of the luminescence remains undetermined but evidence will be presented below that in *Digaster* it is not defensive. It will also be shown to suggest involvement of a peroxidase system.

Observations on luminescence in *Digaster keasti* have been restricted to a preliminary stage pending detailed investigation of this phenomenon in Australian earthworms and because of the necessity for early

fixation of the single specimen available for the present study to ensure that its condition was suitable for taxonomic description.

In Vivo Observations

The undisturbed worms showed no luminescence to the dark-adapted eye nor was luminescence visible after exudation of coelomic fluid from the dorsal pores following stimulation mechanically (by squeezing and tapping) or electrically (by application of electrodes of a 50 volt magneto).

In Vitro Observations

Copious exudiation of coelomic fluid into a glass vial was induced by electrical stimulation. The exuded coelomic fluid showed no luminescence. On addition of excess of 0.022M solution of H_2O_2 in distilled water a 'flash peak' of luminescence was immediately obtained followed by a decline of luminescence to zero over a few minutes. During the period of decline further lesser peaks of luminescence were obtained by shaking the solution, presumably as a result of mechanical lysis of further coelomocytes.

Pipetting dilute or concentrated peroxide onto the surface of the hitherto non-luminescent worm also evoked exudation and luminescence.

Luminescence of the *in vitro* coelomic fluid and the peroxide stimulated worm was of only moderate brightness to the dark-adapted eye and had no perceptible colour.

Similar experiments on new material of *D. brunneus* from Gin Gin (QM G8483) revealed no luminescence.

Conclusions

A capacity for luminescence of the exuded coelomic fluid of *Digaster keasti* has been demonstrated. The fact that luminescence, though evoked by experimental addition of hydrogen peroxide, is not evident in the untreated exudate militates (at least for this species) against the view of Gilchrist (1919) that luminescence is a defense against predators. Under what natural conditions *Digaster* produces luminescence is unknown and awaits demonstration in further material.

Production of luminescence on addition of H_2O_2 to the exudate is compatible with, though not definite proof of, the operation of a peroxidase system as described for *Diplocardia longa*. It is provisionally assumed that luminescence on addition of H_2O_2 was not merely the result of lysis of coelomocytes by the peroxide as sufficient lysis might be expected from mechanical and electrical excitation of the fluid. Demonstration of the peroxidase system nevertheless requires further confirmation and the component of the coelomic exudate (coelomocyte or fluid) in which luminescence occurs has yet to be localized. For the present it is assumed that the site is the coelomocyte.

If as suggested by Bellisario *et. al.* (1972) peroxide is normally generated by the action of oxidase in the coelomic fluid, it is not clear why endogenous peroxide thus or otherwise generated does not cause spontaneous luminescence given sufficient lysis of coelomocytes, in *Digaster*. The possibility appears worthy of investigation that control mechanisms exist which restrict peroxide production to such time as luminescence is required. As the body wall is opaque it would appear that the appropriate conditions include exudation of coelomic fluid. As luminescence is not produced when it might be defensive, it seems conceivable that it may be triggered where intraspecific recognition is required as in copulation as suggested by Flaugergues as long ago as 1771 (*fide* Harvey, 1952). Such exchange of light signals would represent a simple approach to the highly controlled, more sophisticated exchanges which some polychaetes have developed from simpler forms of light emmission in their class. The simplicity of the photo-receptors of earthworms would seem compatible with a simple level of light production.

Failure to reach a consensus on any one suggested function of luminescence in earthworms may be due to failure to recognise the possibility of diverse functions among the different species. Nevertheless bioluminescence of direct biological significance presumably was preceded in evolution by production of luminescence with no distinct function, as a by-product of other metabolic processes and the later selection for luminescence in response to some adaptive advantage which it conferred. The possibility cannot be ruled out that bioluminescence still persists as a functionless biproduct of other metabolic processes, at least in those oligochaetes in which it is not produced immediately and intensely on stimulation or continuously as in *Microscolex*.

The fact that some non-luminescent earthworms contain a luciferin which luminesces in the presence of luciferase of *Diplocardia longa* (*fide* Wampler in *litt.*) suggests that production of potentially luminescent agents (luciferins) probably always preceded development of luminescence and that luminescence may have resulted from the development of the additional factor (luciferase), the primary function of which may not have been the production of luminescence.

ACKNOWLEDGEMENTS

Thanks are due to Professor Allen Keast for donating the material which is the subject of this paper and to Dr John Wampler, Bioluminescence Laboratory, University of Georgia, for critical discussion. The work was supported by an ARGC Grant.

REFERENCES

BELLISARIO, R., SPENCER, T. E., AND CORMIER, M. J., 1972. Isolation and properties of Luciferase, a Non-Haeme Peroxidase, from the Bioluminescent Earthworm, *Diplocardia longa*. *Biochemistry* **11**(12): 2256–2266.

GILCHRIST, J. D. 1919. Luminosity and its origin in a South African earthworm (*Chilota* sp.) *Trans. R. Soc. S. Afr.* **7**(3): 203–212.

HARVEY, E. N. 1952. Bioluminescence. (Academic Press: New York).

JAMIESON, B. G. M. 1971. A review of the Megascolecoid Earthworm Genera (Oligochaeta) of Australia. Part I—Reclassification and checklist of the Megascolecoid Genera of the world. *Proc. R. Soc. Qd*, **82**(6): 75–86.

1975. The genus *Digaster* (Megascolecidae: Oligochaeta) in Queensland. *Mem. Qd Mus.* **17**(2): 267–292.

SPENCER, W. B. 1900. Further descriptions of Australian Earthworms, Part I. *Proc. R. Soc. Vict.* **13**(1): 29–67.