A NEW SPECIES OF *DERO* (*ALLODERO*) (NAIDIDAE: OLIGOCHAETA) INHABITING THE WOLFFIAN DUCTS OF THE GREEN TREE FROG (*LITORIA CAERULEA*) IN QUEENSLAND

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A new species of naidid oligochaete, *Dero* (*Allodero*) *litoria*, inhabiting the ureters of the Green Tree Frog (*Litoria caerulea*), is described from Queensland. This is the first record of this subgenus from Australia. The new species is most similar to *Allodero hylae* (Goodchild, 1951) of SE USA, from which it is distinguished by having gills within a branchial fossa and mid-segmental dorsal ridges which extend around to the ventral setae. ☐ *Oligochaeta, Naididae, new species, amphibia, commensalism, Queensland, Australia.*

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Dero Oken, 1815 (Naididae) is diagnosed by a fossa (cavity) around the anus, which usually contains gill filaments. Three subgenera, *Aulophorus*, *Dero* and *Allodero* are usually recognised. The first two are very similar: both have ventral setae that are longer and thinner on anterior segments than on posterior segments, a full complement of dorsal setae, a well developed branchial fossa and gills and all species are free-living. They differ from each other only in that *Dero* lacks the pair of elongate palps found on the posterior border of the *Aulophorus* fossa. By contrast, *Allodero* is mostly endocommensal in frogs, have all ventral setae similar and (especially in commensal specimens) the dorsal setae and branchial organs are often reduced or absent.

There are seven species in *Allodero*. Five of these, *A. bauch-iensis* (Stephenson, 1930), *A. hylae* (Goodchild, 1951), *A. lutzi* (Michaelsen, 1926a), *A. floridana* Harman, 1971, and the new species, are known to be endocommensal in frogs, although at least one (*A. lutzi*) can exist in a free-living state. A sixth, *A. malayana* (Stephenson, 1931) has so far only been found free-living.

The new species described in this paper is the first record of the subgenus in Australia, although the other two subgenera have been recorded previously (Brinkhurst 1971; Pinder & Brinkhurst, 1994). It is only the second species of Naididae to be recorded only from Australia, the other being a species of *Slavina* Pinder & Brinkhurst, 1998). The other 29 species recorded in Australia (Pinder & Brinkhurst, 1994) also occur in other continents.

Discovery of the new species of *Allodero* was an incidental finding made during dissection of Green Tree Frogs (*Litoria caerulea*). The frogs had been submitted to the Rockhampton Veterinary Laboratory by a farmer who wished to know why so many of this species were found in poor condition in her locality. All worm specimens have been deposited with the Queensland Museum (QM).

Very few oligochaetes have taken to a symbiotic lifestyle compared to the other major clitellate group, the leeches. Gelder (1980) reviewed symbiotic relationships between 13 species of Enchytraeidae, Naididae and Haplotaxidae and their hosts (worms, molluscs, crustaceans and fish). In addition, three Phreodrilidae of the genus *Astacopsidrilus* have been found to be ectocommensal on *Euastacus* crayfish in Australia (Goddard, 1909; Brinkhurst, 1991; Pinder & Brinkhurst, 1997). Most relationships appear to involve ectocommensalism, with endocommensalism and endoparasitism restricted to *Allodero* and another naidid *Chaetogaster limnaei* von Baer, 1827 (Gelder, 1980). The cosmopolitan *C. limnaei* is endo- or ectocommensal, or even parasitic (depending on the subspecies) on molluscs. In Australia, it has been found to inhabit the pulmonary chamber and body surface of several lymnaeid and planorbid gastropods (Davies, 1913).

MATERIALS AND METHODS

Between Feb., 1996 and Jan., 1997 the farmer submitted a total of 52 Green Tree Frogs (27 ♂♂ and 25 ♀♀) to the laboratory to establish a reason for their apparent ill health. The number of frogs collected and the frequency of the submissions was determined only by the time available to the farmer. Soon after collection they were brought to the laboratory, sacrificed by pithing and then dissected to remove various internal organs for examination. The kidneys and Wolffian ducts were dissected free and placed in a petri dish containing phosphate buffered saline (pH 7.1). Using a dissecting microscope, fine scissors and forceps the Wolffian ducts were opened along their length. Oligochaetes were removed and placed in 70% alcohol. Tissues intended for histological examination were fixed in 10% buffered neutral formalin, embedded in paraffin and stained with haematoxylin and eosin using routine methods.

Alcohol preserved specimens were either mounted in CMCP-10 or stained in Grenacher's Borax Carmine, then cleared in methyl salicylate and mounted in Permount.

The following abbreviations are used in the figures. *Allodero lutzi*: b=brain, bf=branchial fossa, bu=buccal canal, c=coelomocytes, cu=cuticle, cg=chlorogogen tissue, e=epidermis/epidermal cell, g=gill, i=intestine, m=mouth, mu=body wall muscle, n=nerve cord, p=pharynx, pg=pharyngeal gland tissue, rm=retractor muscles. *Litoria caerula*: le=lumenal epithelium of wolffian duct, kp=kidney parenchyma.

SYSTEMATICS

Phylum ANNELIDA
Class OLIGOCHAETA
Family NAIDIDAE
Dero Oken, 1815
Dero (Allodero) litoria sp. nov.
(Figs 1-4)

MATERIAL. HOLOTYPE: QMG213863. Immature on slide, found on Green Tree Frog (*Litoria caerula*) collected on farmland, Mt Larcom, 23.87°S 150.91°E, central Queensland, between ii.1996 and i.1997, coll. H.A. Luckey. PARATYPES: QMG213864 - 213869, 213871 - 213874 and 213877 (11 slide mounted immatures); 16 alcohol specimens (in three vials), QMG213870, 213875 and 213876. Collection details as for holotype.

DESCRIPTION. Dimensions (preserved and slide mounted) 3.2mm × 170µm (49 segments) to 6.2mm × 350µm (79 segments). Colour reddish-brown when alive. Anal end of some specimens without setae and not clearly segmented while anal ends of other specimens terminating abruptly and obviously incomplete, but with intact (or almost intact) body wall, indicating recent regeneration and possibly asexual reproduction by fragmentation. Budding not evident. A ring of thickened body wall occurring towards the posterior of each segment (Fig. 1C), creating a ring level with the setae. This is most noticeable dorsally (Fig. 1A) and at the ventral setal bundles, so that the dorsal setae occur on a ridge and the ventral setae appear to protrude from papillae, and is least developed ventrally. This ring is created by enlargement and bunching up of the epidermal cells (Fig. 1C).

Alimentary canal difficult to make out and possibly not continuous in some specimens. Generally, a thin walled buccal canal (Fig. 1A) leads from mouth to a thickened pharynx in III and IV, with muscular attachments to dorsal body wall of III. Pharyngeal gland tissue present in III to IV or V (Fig. 1A). Lumen in gut very narrow (Fig. 1A) or not visible. No gut contents visible. In one specimen, which has a prostomium and brain, so is otherwise developed anteriorly, there appears to be no mouth, buccal cavity and pharynx. Posterior gut often difficult to trace but (in fully regenerated specimens) dorsal anus present on the last segment. Gut and dorsal blood vessel surrounded by masses of brown chlorogogen tissue from VI onwards (Figs 1A, 3), which often fills the entire coelomic cavity of some segments. Chlorogogen cells elongate tear-drop shaped, arising from the wall of the intestine, with a large deeply staining nucleus and coarsely granular cytoplasm. Sub-spherical coelomocytes present, with cytoplasm more transparent than that of the chlorogogen cells. Coelomocytes (Fig. 1A) either free or lying adjacent to the chlorogogen or body wall, varying in density from sparse to filling the coelom between the body wall and chlorogogen. Some specimens with a branchial fossa, varying in development from barely apparent to an obvious chamber. In two specimens the fossa contains two pairs of short, rounded, knob-like gill filaments, not protruding from the fossa (Fig. 1B). The gut and branchial fossa appear to be most developed when the dorsal setae are also most numerous.

Bifid ventral setae from II (Fig. 2A-C), 4 to 6 per bundle anteriorly, usually 3 or 4, rarely 2 or 5 per bundle posteriorly, most 72.5-92.5µm long with a slightly distal nodulus. Setae of II-VI (Fig. 2A,B) not especially longer and thinner than those in following segments (Fig. 2C), although the most posterior setae often slightly shorter

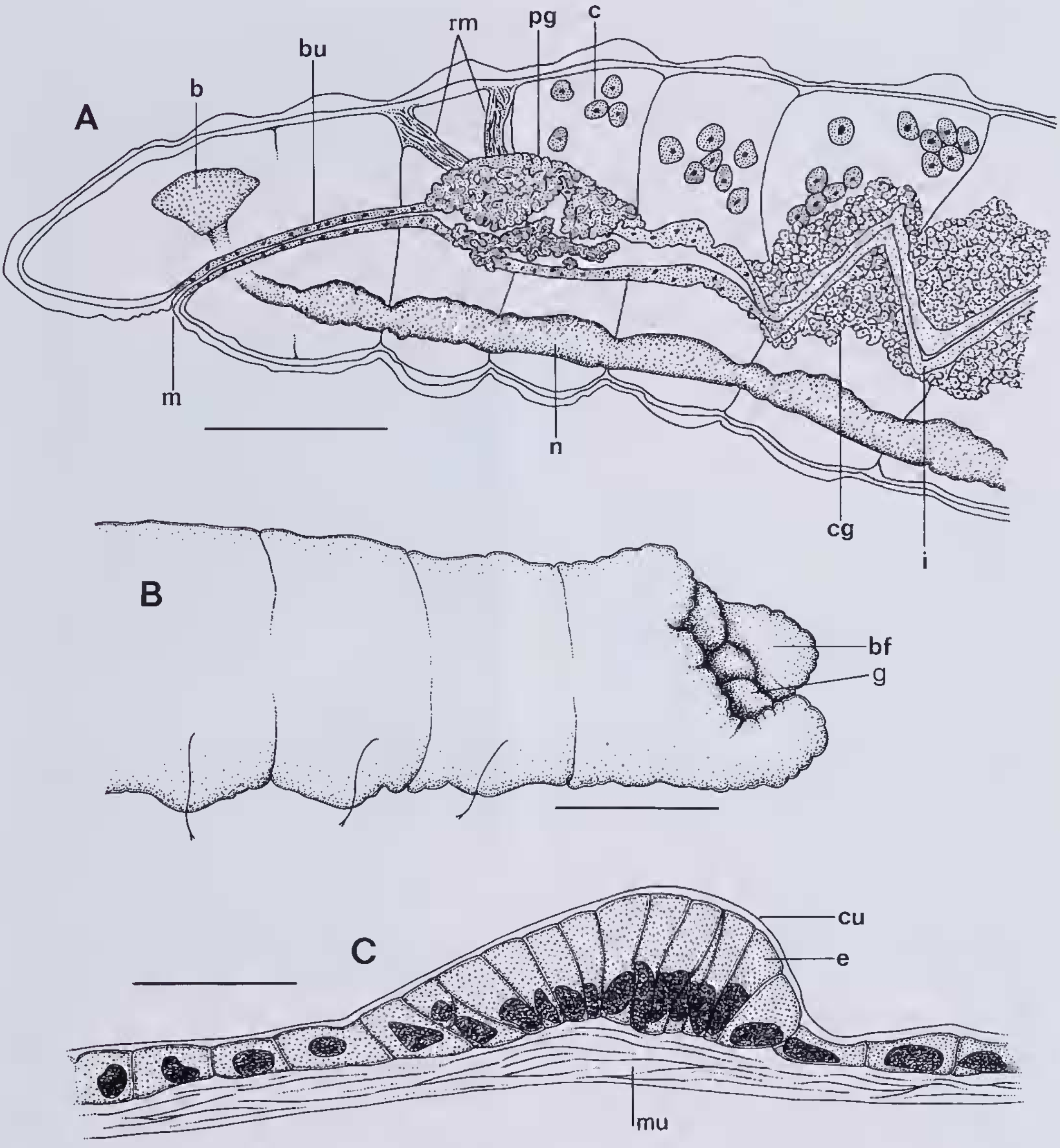

FIG. 1. *Allodero litoria* sp. nov. A, anterior seven segments, based on paratype G213865 with details from other specimens; B, posterior end of holotype showing three of the four gills, the fourth is obscured by the dorsal wall of the fossa; C, section of dorsal body wall of paratype G213867 showing ridge created by enlarged and bunched epithelial cells. Scales: A=100µm, B=80µm, C=20µm.

(62.5-70µm) and the nodulus slightly more distal anteriorly. Dorsal setae from IV, often absent in some segments or absent entirely in some specimens. Each bundle with 1 or 2 needles (55-73µm long) with a distal nodulus, bifid tips with short curved outer teeth, the lower tooth much thicker and somewhat longer than the upper, often with a third very small intermediate tooth (Fig. 2D, E). One short hair (95µm long) present in one bundle of a single specimen, accompanied by 2 needles (Fig. 2F).

Worms found within the ureters of *Litoria caerulea* (Figs 3, 4).

DISTRIBUTION. *Allodero* is circum-tropical (Africa, SE Asia, Central and South America and

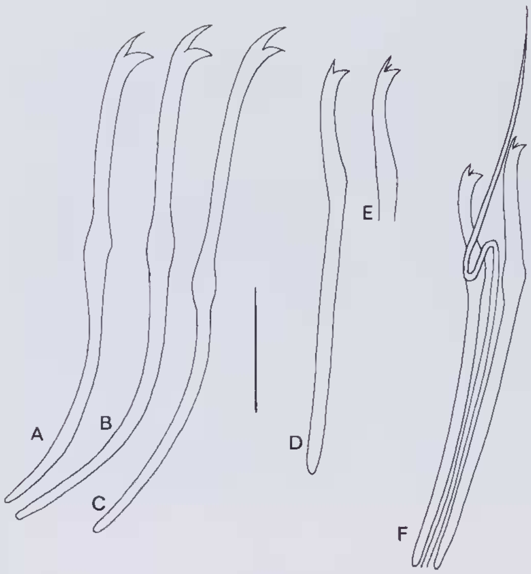

FIG. 2. *Allodero litoria* sp. nov. A, ventral seta of III; B, ventral seta of V; C, ventral seta of a posterior segment; D, dorsal needle with bifid tip; E, tip of dorsal needle with intermediate tooth; F, hair and needle setae of XVI of paratype G213864. Scale = 20μm.

SE North America). The new species from Mt Larcom (23.87°S 150.91°E) fits this pattern. Full distribution of the species in Australia has not been determined. The frog host species is widely distributed in N and E Australia. Host frogs were found several hundred metres from the nearest water (farm dams).

ETYMOLOGY. For the frog host.

REMARKS. The presence of a gilled branchial fossa places the new species in *Dero*, while the endocommensal habit, uniform ventral setae and a tendency to lose the dorsal setae and fossa indicates its placement in the subgenus *Allodero*.

The new species is most similar to *A. hylae*, which inhabits the ureters of various species of *Hyla* (Hylidae) in SE USA (Harman & Lawler, 1975). The setae of *A. hylae* are of similar size and form to those of *A. litoria*, except that the needles of the former are usually one per bundle (one or two in *A. litoria*) and do not have intermediate teeth. Intermediate teeth are otherwise known only from *A. floridiana* but in that species they are much more pronounced (Harman, 1971). However, the presence or absence of intermediate teeth on bifid setae is considered a poor character for species separation in *Dero* and other naidids (Grimm, 1987). *A. hylae* also has papillae at the position of the dorsal setae and the illustrations of Goodchild (1951) show these to be similar to the ridges of *A. litoria*, except that those of *A. hylae* do not extend to the ventral setae according to Goodchild (1951). Another significant difference between *A. hylae* and the new species is that the *A. hylae* specimens observed by Goodchild (1951) and Harman & Lawler (1975) had only a rudimentary fossa (where present at all) and none had gills. It is possible that *A. hylae* would develop gills in a free-living state, but no specimens of this species have ever been found outside of the frog hosts, despite a search in ponds frequented by the frogs (Harman & Lawler, 1975). The only other *Allodero* known to have gills within the branchial fossa is *A. lutzi* of South America which has four pairs, as opposed to the two pairs of *A. litoria*. In *A. lutzi*, the gills are lost when the worms are within the frogs. Neither gills nor papillae have been reported for the remaining three species (*A. bauchiensis*, *A. floridiana* and *A. malayana*). Only a few specimens of the new species had fossa and gills which may indicate they had been within the frogs for a shorter period.

The apparently incomplete alimentary canal of many *A. litoria* specimens has also been noted for all other commensal species of *Allodero*, including *A. hylae* (Goodchild, 1951), *A. bauchiensis* (Stephenson, 1930), Brazilian specimens of *A. lutzi* (Michaelsen, 1926b) and *A. floridiana* (Harman, 1971). This suggests that the worms do not ingest food while in the frog hosts, although Stephenson (1930) suggested that *A. bauchiensis* absorbed secretions of the frogs lachrymal gland through its body wall. Gelder (1980), in a review of oligochaete associations, discussed what is known of *Allodero* feeding biology, but did not mention the chlorogogue tissue surrounding the intestine that is particularly abundant in many *Allodero*, compared to other *Dero* and other Naididae. This tissue, which often fills the coelomic cavity of *Allodero*, is known to be involved in storage and metabolism of glycogen and lipids (Jamieson, 1981). The worms may use the chlorogogue tissue to build up an energy store while feeding outside of their hosts: the gut then degenerating when the worms are inhabiting the frogs. At least one species, *A. lutzi*, is known to occur outside of its frog hosts, in aquatic habitats, where it develops gills and dorsal hairs and presumably then feeds in the normal way. How and when the worms find and enter new hosts is not known. Non-commensal *Dero* are all aquatic and frogs may be infected when in water, which they enter to lay eggs.

The frogs, which were subjectively judged by the farmer to be thin compared to the general popula-

FIG. 3. Cross section through *L. caerula* Wolffian duct with enclosed specimen of *A. litoria* sp. nov.

tion, had a mean body length (SVL) of 8.0cm, ranging from 6.5 to 10cm. The new species of *Allodero* was found associated with slight to moderate dilatation of one or both Wolffian ducts in 12 frogs. The number of worms removed from the ducts of each frog is as follows (with the number of frogs in parentheses): 1(1), 2(2), 3(2), 5(1), 6(1), 10(1), 11(1), 12(1), 15(1), and 23(1). The cloacas were not examined. While the ducts were being dissected, the oligochaetes were observed to actively move to the proximal portions adjacent to the kidneys. The worms appeared to be approximately evenly distributed between left and right ducts.

Most of the 52 frogs submitted for examination were thin and had depleted fat bodies. Regardless of the presence or absence of oligochaetes no inflammatory or degenerative lesions were detected on histopathological examination of the kidneys and Wolffian ducts (Figs 3, 4). Histological examination of other organs revealed inflammatory lesions in the livers of 34 frogs, with the most severe forms associated with the presence of *Myxidium* trophozoites in the hepatic ducts (Hill et al., 1997). The livers of the 12 frogs which harboured oligochaetes in their Wolffian ducts were normal in four frogs while the remainder had hepatitis of varying severity.

Apart from possible partial obstruction of the Wolffian ducts this new species of *Allodero* appears to have no adverse effect on the host. In this respect it is similar to the other endosymbiont oligochaetes, *A. lutzi* (Michaelsen, 1926a) and *A. hylae* (Goodchild, 1951), described in the Wolffian ducts of the amphibian genus *Hyla*. Following the discovery by Markiw & Wolf (1983) that *Myxosoma cerebralis*, the agent of salmonid whirling disease, required a tubificid worm in its life cycle, Delvinquier (1986) speculated that *Myxidium* in amphibians may also require a similar intermediate host. Whether any relationship exists between the new species of oligochaete described here and the *Myxidium* described by Hill et al. (1997) is presently unknown but is under investigation.

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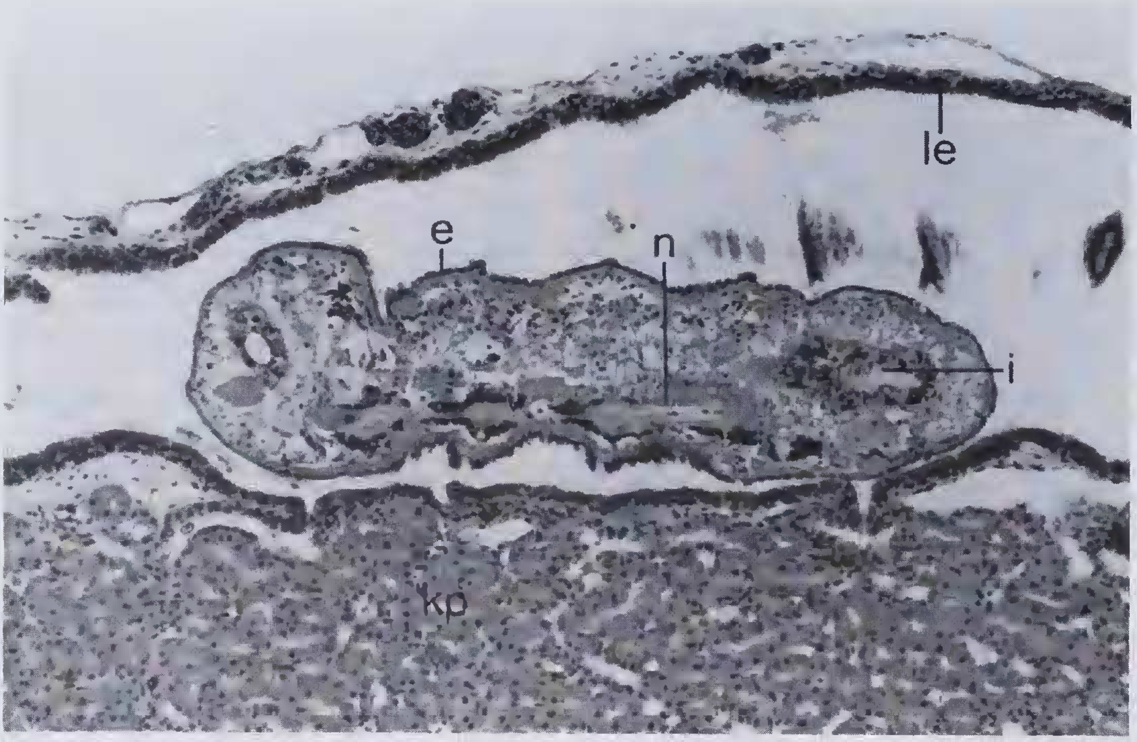

FIG. 4. Cross section through *L. caerula* kidney and Wolffian duct with enclosed specimen of *A. litoria* sp. nov.

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