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Eco-taxonomic profile of an iconic vermicomposter — the 'African Nightcrawler' earthworm, *Eudrilus eugeniae* (Kinberg, 1867)

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ABSTRACT

Eudrilus eugeniae (Kinberg, 1867), an adaptable exemplar of an anatomically advanced earthworm having direct fertilisation, is reviewed ecologically. A tropical West African species originating in savannah soils, it thrives on organically rich substrates. It has a rapid life-cycle from cocoon to maturity in as little as 47 days. Presence of this worm raised experimental pasture yields up to 83.9%. Copious pellet-like casts deposited onto the soil surface are sought by roots. Passage of organic material through its gut reduces microbial pathogens and the resulting vermicompost product has enhanced nutrients, and microbial and enzymatic properties. Preliminary pharmaceutical reports are of inhibition of 'Golden staph' *Staphylococcus aureus* and 'Thrush' *Candida albicans*, plus anti-tumour effects in cancer cell lines. Its handling characteristics make this worm highly suitable for vermiculture with ecological and economic provisioning of: (1) fishing bait or 'seed' cultures, (2) high-protein worm biomass for stock feeds, (3) organic fertiliser, (4) bio-stabilisation of contaminated matrices/fluids, (5) recycling of organic 'wastes', (6) carbon sequestration in soil organic matter (SOM, or humus), (7) bio-prospecting for pharmaceuticals, cosmetics or 'silk', and (8) eco-toxicology/ethology research. New reports are of cultivation in Denmark, South Africa, Egypt, Saudi Arabia, Ecuador, Peru, Indonesia, Malaysia, Thailand and Vietnam. *Eudrilus eugeniae* is figured and its ecological profile, global distribution and taxonomy updated with mtDNA barcodes.

KEY WORDS: Annelida, Oligochaeta, Eudrilidae, vermiculture, DNA barcoding, soil ecology, megadrile systematics.

INTRODUCTION

This paper reviews the current knowledge of the eco-taxonomic and morpho-molecular profile of *Eudrilus eugeniae* (Kinberg, 1867). It is not known when its vermiculture potential was initially recognised but its initial wide 'expat' distribution has been mainly attributed to accidental human transportation, since it was already well established in European colonies in the South Atlantic, Caribbean, Indian and Pacific Oceans from the early 1800s (e.g. Perrier 1872; Michaelsen 1900, 1903; Stephenson 1923). More recently it has been deliberately introduced for commercial and experimental purposes. Ecological studies commenced from the mid-1960s to the late 1980s (e.g. Eno 1966; Madge 1969; M'ba 1978; Neuhauser *et al*. 1979; Graff 1981; Bano & Kale 1988). This species does not conform to standard earthworm ecological classifications such as those of Lee (1959) as reported by (Blakemore 2008*b*) since it responds facultatively, spanning the spectrum as either a geophage 'topsoil' species or alternatively as a detritivore 'litter' species. Lee (1985) characterised it as a topsoil species and it is known to deposit numerous nutrient-enriched casts on the surface of the soil — equivalent to 156.8 t ha^{-1} per annum according to Gates (1972: 52) — as well as producing uniformly enhanced vermicomposts when reared on diverse organic 'waste' substrates.

In response to frequent specimen identification requests, the taxonomy of *Eudrilus eugeniae* is also reviewed and supported with mtDNA COI barcodes (see Appendix). Although some GenBank data are available, this is the first genetics report coupled with a detailed species description. Further data is presented from comparative ecological

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studies conducted by the author (Blakemore 1994, 1997, 2008*a*). This update notes the confusion over reproductive and digestive organs, counters misdescriptions (such as that by Vijaya *et al.* (2011), who mistake vital segmental counts), and further fixes distribution reports, such as New Zealand by Sims and Gerard (1985, 1999). A summary of the global distribution for *Eudrilus eugeniae* is updated from Michaelsen (1900, 1903). New ecological data is consolidated.

MATERIAL AND METHODS

Earthworms from worm farms in Australia and the Philippines were studied, and identities confirmed on Ghanaian and Indonesian samples. Taxonomy follows the codes and conventions of Blakemore (2012*b*). PCR methods similar to those described in Blakemore *et al.* (2010) were used for mtDNA barcoding. Results of genetic analyses with BLAST programs (www.blast.ncbi.nlm.nih.gov/BLAST.cgi) are compared to Genbank (genbank.com) in the Appendix. Laboratory and glasshouse experiments plus field trials were conducted with 28 earthworm species including *Eudrilus eugeniae* by Blakemore (1994, 1997, 2008*a*) with extensive literature searches; herein data is integrated and compared with recent published reports. Abbreviations: ANC = African Nightcrawler; np = nephropores; Qld = State of Queensland in tropical NE Australia; rhs/lhs = right/left hand side.

TAXONOMY

Phylum Annelida Lamarck, 1802
Class Oligochaeta Grube, 1850
Order Megadrilacea Benham, 1890
Family Eudrilidae Claus, 1880

Eudrilus eugeniae (Kinberg, 1867)

Figs 1–4

Lumbricus eugeniae Kinberg, 1867: 98. [Type locality: Humid mounts and valley of St Helena (15°56'S 05°43'W). Types in Natural History Museum, London BMNH 1904.10.5.550 with Swedish Museum label: "*Lumbricus Eugeniae Kinberg St Helena Swed. State Museum*." The specimen was in moderate condition when briefly inspected in June, 2013 (Blakemore 2014: 122)].

Eudrilus decipiens Perrier, 1871: 1176; 1872: 78, figs 26–30; Horst 1887: 247 (syn.: *lacazii, peregrinus, boyeri*). [From Antilles. Types in Paris].

Eudrilus lacazii Perrier, 1872: 75. [From Martinique (collected 1826). Types in Paris].

Eudrilus peregrinus Perrier, 1872: 77, fig. 76 (of ova). [From Rio de Janeiro (collected 1833). Types in Paris].

Eudrilus boyeri Beddard, 1886: 302. [From New Caledonia. Types BMNH 1904:10:5:612].

Eudrilus sylvicola Beddard, 1887: 372. [From British Guyana. Types BMNH 1904:10:20:408].

Eudrilus jullieni Horst, 1890: 225. [From Liberia. Types in Leiden?].

Eudrilus roseus Michaelsen, 1892: 224, fig. 10. [From Caracas, Venezuela. Types Humboldt Museum, Berlin 2162. Michaelsen notes "?*Eudrilus perigrinus* E. Perr." (sic)].

Eudrilus erudiens Ude, 1893: 71. [From Bermuda. Types?].

Eudrilus eugeniae: Beddard 1895: 604, fig. 30 (syn.: *lacazii*, *peregrinus*, *decipiens*, *boyeri*, *sylvicola*, *jullieni*, *roseus*); Eisen 1900: 135, figs 27–50, 95–97; Michaelsen 1900: 402 (syn.: *decipiens*, *lacazii* + *peregrinus* Perrier, 1872; *boyeri*, *sylvicola*, *jullieni*, *roseus*, *erudiens*); Stephenson 1923: 486; 1930: 873; Gates 1942: 137; 1972: 51; 1982: 72; Sims & Gerard 1999: 146, fig. 52; Sims 1987: 386; Csuzdi & Pavlicek 2009: 13 (excluding the *peregrinus* synonym by oversight?); Blakemore 1994; 2002; 2012*b*; 2013; 2014: 122.

Etymology: Named after Johan Gustaf Hjalmar Kinberg's Swedish survey ship, the 'Eugenie'.

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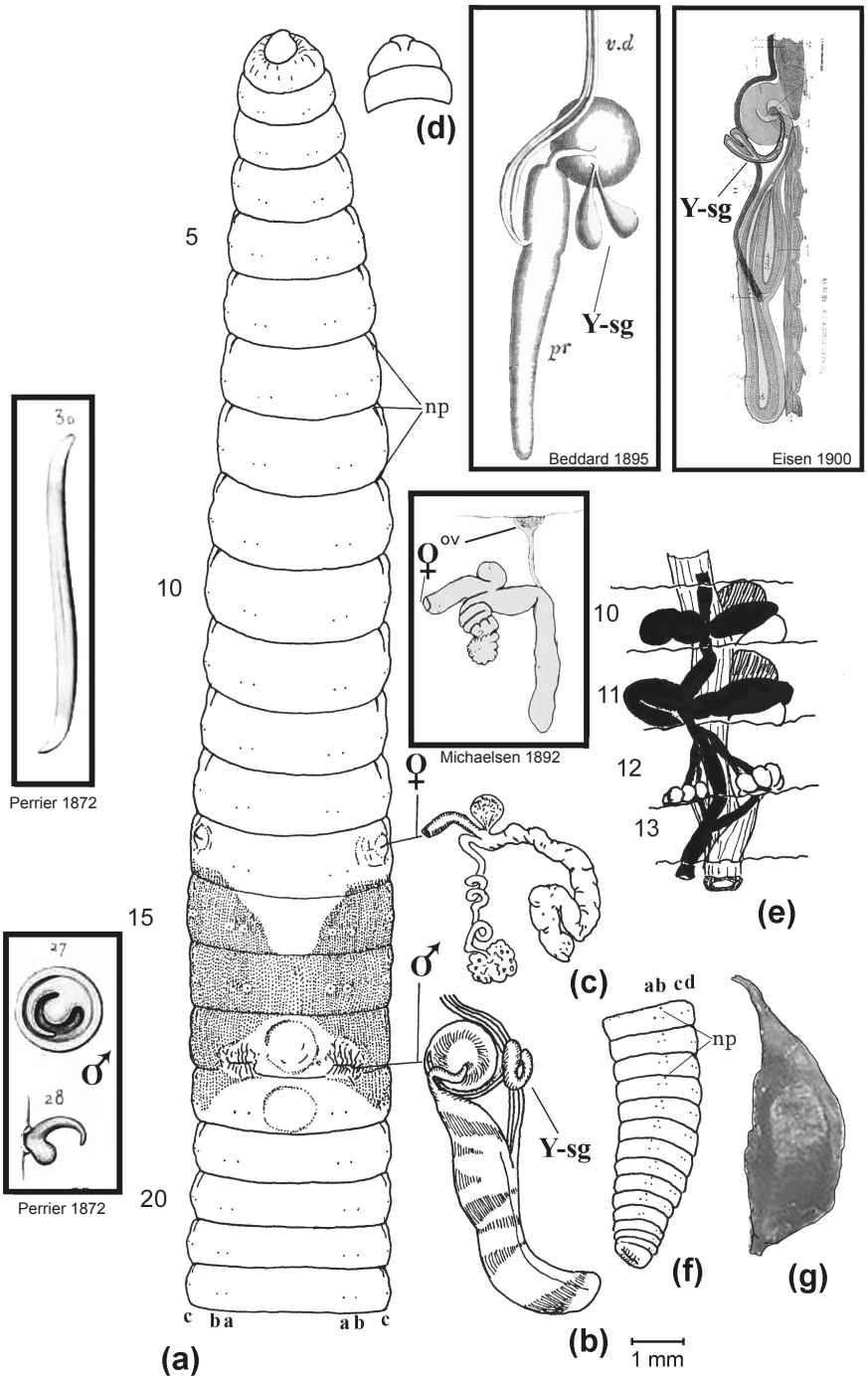
Fig. 1. A *Eudrilus eugeniae* specimen from a vermicompost site.

Description:

External morphology:

Body length: Complete matures, 90–185 mm (pers. obs. and Gates 1972) or up to 250–400 mm under optimal culture conditions (Viljoen & Reinecke 1994; Parthasarathi 2007); posterior tapering, becoming thinly flattened in terminal 'zone of growth' (Gates 1982). *Width*: Approximately 4–8 mm.
Mass: Mean per adult *ca.* 1.0 g (pers. obs.) or optimal maximum 5.0–6.0 g.
Segments: 161–211 (pers. obs. and Gates 1972) or 250–300 (Viljoen & Reinecke 1994, suggesting that larger worms add segments); constriction of 40–46 seen in several Qld specimens may be artefactual.
Colour: Red-brown dorsum fading posteriorly; anterior with bright blue/green iridescent sheen from cuticle diffraction, ventrum beige, clitellum darker (sometimes lighter) than surroundings.
Prostomium: Small, open epilobous.
Dorsal pores: None.
Setae: Eight per segment from 2, closely paired; setae a–b on 17 absent (dehisced); ratio of aa:ab:bc:cd:dd:U on 7=6:1:5:1:10:0.5. Penial/genital setae absent.
Nephropores: Just behind anterior furrow of each segment (longitudinal slits) from 3/4 in c lines or slightly more median (sometimes in d lines).
Clitellum: 13, 14, 15–18, usually 13, 14–18 and interrupted ventrally.
Male pores: In 17 on tips of longitudinally grooved, tapering, eversible penes in large ventral chambers, retracted as lateral slits with wrinkled lips just anterior to 17/18 in line with b setae.
Female pores: Combined with modified 'spermathecal pores' (see Fig. 2) lateral, presetal in 14 as raised intrasegmental openings just anterior to c setae. Gates (1972: 51) calls these "vaginal apertures".
Genital markings: Central raised pad centred in 17 between male pores, faintly repeated in 18; sometimes undeveloped or as elliptical, opaque area in 16–18 (Gates 1982).

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Internal anatomy:
Septa: From 4/5; (6/)7/8/9 and 14/15 thickened.
Dorsal blood vessel: Single, truncated before anterior hearts in 7; according to Gates (1972: 51) connects to paired supra-oesophageals in 7–14 and paired extra-oesophageals median to the hearts.
Hearts: In 7 lateral, in 8–11 latero-oesphageal, all distended with blood in some Qld specimens (cf. Gates (1972) who said the anterior hearts were undistended).
Gizzard: Weakly muscular in 5 immediately behind pharyngeal mass.
Calciferous glands: Ventral spheroidal sacs in 10 and 11 (concealed by seminal vesicles): large and pink due to blood supply with many internal lamellae; also in 12 (concealed by seminal vesicles) a pair of yellow, lobular 'calciferous' glands which are medially placed lateral to the oesophagus and ducted posteriorly into it in 13. This latter pair supplied by quite large blood vessels (from supra-oesophageal vessels). Michaelsen calls the median oesophageal sacs "chylustaschen" but Stephenson (1930) only called the paired glands in 12 "calciferous". Eisen (1900: 138) found neither crystals nor lime granules in the paired "diverticles" in 12, whereas Gates (1972: 51), after claiming calcareous granules in both median and paired glands, classed them all as calciferous.
Intestine: Origin in 14 or close to 14/15. Caeca and typhlosole absent. Small, supra-intestinal glands present in eight to forty-two segments in some of 62–132 (Gates 1972: 52; 1982: table 8) may assist digestion and/or be implicated in the immune competency of the worms.
Nephridia: Paired, large coiled holonephridia in each segment from 4, not obviously vesiculate.
Male organs: Holandric with two large, unpaired (or attached?) sacs seen ventrally in 10 and 11, each contain a testis anteriorly and funnels posteriorly, i.e. two pairs of testes in 10 and 11; paired seminal vesicles occupy 11 and 12 and are filled with coagulum. The testes funnels are small and free from iridescent spermatozoa which aggregate in the ducts and thus are easily missed. The male apparatus is complicated and descriptions differ somewhat; the copulatory chamber contains a pointed and curved penis plus a large round papilla or porophore of what Eisen (1900: 140, figs 44, 46) and Gates (1972) describe as a "Y-shaped gland" that opens into a groove going nearly to the tip of the penis. Eisen found the product of this Y-shaped gland to be a secretion similar to that of the silk gland of a caterpillar (possibly analogous to penial setae as found, for example, in *Nsukkadrilus mbae* Segun, 1977, to remove sperm of previous concopulant?). The Y-shaped gland is lacking in *Eudrilus pallidus* Michaelsen, 1891 and the copulatory

Fig. 2. *Eudrilus eugeniae*: (a) ventral view of Qld specimen, (b) *vasa deferentia* unite to form the muscular euprostates ducting to the centre of the copulatory chamber (characteristic Y-shaped gland on rhs ducts to lhs), (c) 'spermathecal' aperture and combined oviduct (unravelled) to ovisac opposite saccular gland at junction of duct and 'ampulla' (ovary not shown), (d) prostomium, (e) calciferous glands, hearts and dorsal vessel, (f) dorso-lateral view of caudal segments narrowing to pygomere, (g) cocoon. Boxed are: Perrier's (1872: figs 27, 28, 30) figures of male organs – with penis both retracted and everted – plus an enlargement of a seta (his fig. 29 differs somewhat in its internal organ details); Michaelsen's (1892: fig. 10) figure of female organs also showing ovary "ov" (or ovisac?) on 12/13; plus Beddard's (1895: fig. 30) figure of male organs with glandular appendices to bursa copulatrix sometimes fused to form a "*single horseshoe-shaped*" appendix next to what Eisen (1900: fig. 44) called the silk-producing "*Y-shaped gland*" (indicated as "Y-sg").

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chambers are absent from *E. simplex* Michaelsen, 1913, serving to anatomically separate them from *E. eugeniae* according to Beddard (1895) and Gates (1972: 51).

Female organs: These are complex and difficult to characterise correctly. Large egg-filled ovisacs attach to each spermathecal atrium (although Gates (1942: 142) mistakenly calls this the 'diverticulum') or duct by long, coiled oviduct tubes in 14, sited opposite a saccular gland. Eisen's (1900: 139) description differed from Beddard's (1895) but both (mistakenly?) agreed that ovaries in 13 are combined with ovisacs; and, whereas Eisen thought there were two pairs of ovaries in segment 13, Gates (1972: 52) had the second, functional pair in 14. However, Michaelsen (1892: 225, fig. 10) clearly showed small ovaries paired behind septum 12/13 connecting with the saccular part of the spermatheca (what Sims (1987) calls the "receptaculum seminis") and that the ovisac or "receptaculum ovorum" is terminal to a long second oviduct. Easily missed, this smaller oviduct connection to the spermatheca was figured by Eisen (1900: figs 49–50) and reported by Gates (1942: 142) although Sims (1964: 303, fig. 6) says the small oviduct usually connects with the larger oviduct leading to the ovisac where the eggs mature (as described by Eisen 1900: 139). Histological sections of Vijaya *et al*. (2012) showed a dense mass of sperm in the oviduct they took to confirm internal fertilisation, supporting its classification by Sims (1964) as a "fertilization chamber" rather than a spermatheca. Gates (1972: 51) calls it a vagina whereas Segun (1977: 261, fig. 2) uses the terms "ovo-spermathecal duct" and "ovarian vesicle".

Spermathecae: As just noted under 'Female organs', there is an atrium with muscular sheen in 14 that extends into a long flaccid, convoluted gland, filled with coagulum and enclosed in a sheath; at their junction a long oviduct attaches leading to the ovisac which is opposed by a small saccular outgrowth. The whole or just part of the structure may be referred to as a 'fertilisation chamber' as it functions for internal fertilisation of eggs with sperm, presumably before transfer of the embryos to the cocoon.

Prostates: Large pair of digitiform euprostates, with white muscular sheen from 18 extending to 23; acutely muscular enlargements of loop of paired sperm ducts which attach to apex of copulatory chamber mound centrally. As noted, a smaller blind duct — the Y-shaped gland — attaches to the base of the mound mesially, although Beddard (1895: fig. 30) shows a pair of such glands.

Other internal features: Small saccular 'brown bodies' formed from coelomocytes were observed loose in coelomic cavities from 7 posteriorly; these may enclose shed setal follicles (as also noted by Gates 1972: 52). Beddard (1891: figs 2–3) reported and figured sensory glands in the mid-body that he called "pacinian bodies" which Eisen (1900: 143, fig. 95–97) decided were partly sensory structures to detect sound as "primitive auditory organs" equivalent to otosomes found in *Pontoscolex*; the function in both cases is unknown.

The gut contains soil and/or organic matter (depending on habitat) — this species appears to be an adaptive feeder and will survive in unaltered soil (as noted) but also flourishes on organic material.

Cocoons: Dark coloured with adhesions, tapered lemon-shape with one side usually being flatter, mean size approx. 6×3 mm (from Reineke & Viljoen 1988, who also provide incubation and hatching data); may contain from one to eight hatchlings (Gates 1982).

Distribution (Fig. 3): After Michaelsen (1903: 122); Gates (1942: 98, 1972: 52, 1982: 72): West African origin from Upper Guinea plain or coastal forest including Sierra Leone,

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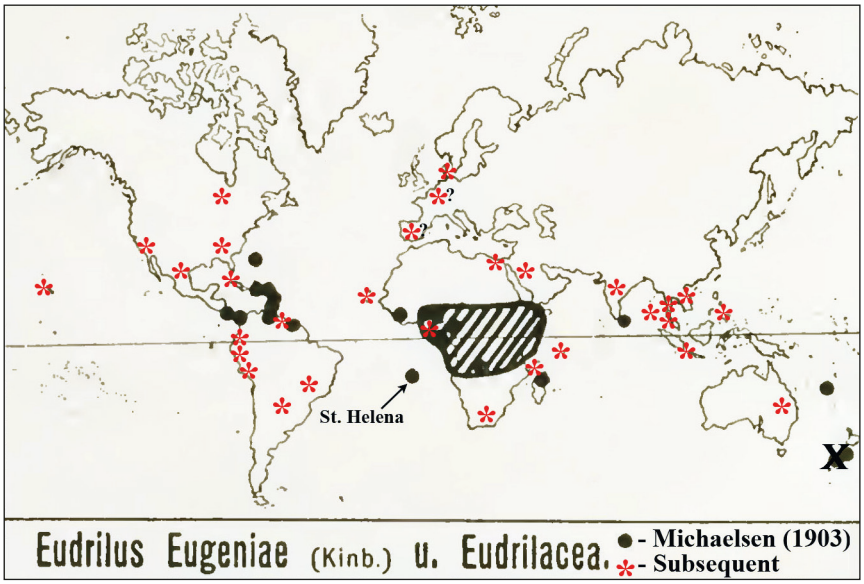


Fig. 3. Distribution map from Michaelsen (1903: chart 1) (hash marks family distribution). Note that New Zealand was in error but other records outside its West African homeland are due mainly to human transportation and the worm's acclimatisation; early Caribbean and Latin American introductions possibly relate to the 16th – 19th century Atlantic slave trade.

Liberia, Ivory Coast, Ghana, Togoland (Benin), Nigeria, Cameroon, Gabon and the Congo; transported and peregrine to many tropical countries such as Madagascar and the Comoros Islands (e.g. Anjouan), Seychelles (Gerlach 2011), Sri Lanka and India (Michaelsen 1903; Stephenson 1923: 486; Dhiman & Battish 2005), and New Caledonia; the Americas: [e.g. Gates (1982: 74) said it owes its North American distribution since the 1950s solely to the fishing bait market having been shipped into every one of the lower 48 United States, such as Florida, Alabama, Georgia, Texas, and even to Hawaii, as well as several Canadian provinces]; Central and South America, e.g. Mexico (Rodriguez-Aragones 1999), Suriname (Horst 1887), Panama [from 1896 — Eisen (1900: 135) said: "Judging from the number of specimens in the collection, this species must be the most common of the large terrestrial earthworms in Panama"], Belize (also as an introduction from the then 'British Honduras' noted by Gates 1982), Venezuela (e.g. *roseus*), Guyana, Colombia (Feijoo *et al*. 2004), Paraguay (Schuldt 2009), Brazil; the Caribbean: e.g. Haiti, Trinidad, Martinique, St Thomas, St Croix, Puerto Rico, Virgin Islands (Michaelsen 1903, 1910; Gates 1942, 1972), Cuba (Gates 1972; Alvarez & Rodriguez-Aragones 2010), Bahamas, Antilles (Gates 1942: 99) and Guadeloupe (Csuzdi & Pavlicek 2009 — who found it in a natural setting indicating it may have become feral there as it is on St Helena); also the Atlantic: Bermuda (as *E. erudiens*), St Helena (type-locality by introduction), Cape Verde (from where it was introduced to New York (Gates 1982), Fernando Po [Bioko] and São Tomé Islands (Michaelsen 1903, 1910). Elsewhere in America, Gates (1982: 72–74) explained in some detail how the first report from the US mainland was in 1950 from "Lake Geneva, Florida" from a "can of worms (bait) inadvertently left behind" and cultured by the camp owner (a Mr

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T. Baker), eventually shipped to all of the USA and Canada where it has been cultured both indoors and outdoors.

The first Australasian taxonomic confirmation was from near Brisbane, Queensland in 1991 (Blakemore 1994, 1999) with stock (surface sterilised cocoons) originally obtained from Canada (Mr G. Bosanquet pers. comm. 1991).

In Europe it was introduced to Hamburg with plants from the West Indies (Michaelsen 1903: 12) and to Kew Gardens in Wardian cases from British Guiana (Beddard 1906). It is rarely reported from northern European glasshouses by Sims and Gerard (1985), albeit rarely, e.g. from Denmark (Blakemore 2007) and eastern Europe, Hungary (Csuzdi *et al.* 2007); also maintained in laboratory cultures, e.g. Vigo, Spain (Dominguez *et al.* 2001).

Plisko (2010) notes that it was deliberately introduced to South Africa (RSA) by Reinecke and Viljoen (1988) from Germany in stock originating in West Africa and that this species is now widely used in RSA farms and is "adapting well to habitats in this country" suggesting its naturalisation there.

Eudrilus eugeniae is stated to be newly introduced to Egypt (Medany & Yahia 2011: 20), but what this paper actually says is: "Four types of earthworms were brought to Egypt from Australia: *Lumbriscus Rubellus* (Red Worm), *Eisenia Fetida* (Tiger Worm), *Perionyx Excavatus* (Indian Blue), and *Eudrilus Eugeniae* (African Night Crawler)". However, *Lumbricus rubellus* Hoffmeister, 1843 has never been proven a vermicomposting worm (Blakemore 1999, 2002), thus it is likely only three species or fewer were involved. *Eudrilus* is newly demonstrated in vermicompost and aquaponics filters in Jeddah, Saudi Arabia (Alamoodi 2014).

At least one worm farmer in Valparaiso, Chile and a technician (Mr Reinaldo Plasencia) in Nicaragua claim to rear *Eudrilus* ("la lombriz africana") sometimes misspelled "*Fudrillus* spp" (Lumbricultura 2014; Monographias 2014), which would both be new national reports. Mr Enzo Bollo Tapia (pers. comm. 2014) communicated that it can be cultivated in Ecuador, Colombia and Peru but that Chile is unsuitable for its survival due to climate, although he did experiment there.

Introduced to the Philippines for vermicomposting in the 1980s, *E. eugeniae* is now distributed in worm-beds on farms over the whole country. A report of its spreading to some mountainous inland areas via agro-forest strips of Negros Occidental by Flores (2007) is unsubstantiated as there is no proof that *E. eugeniae* itself was found. The report just says "*Eudrilus*" based on a novice's key to families. It is also newly reported from Thailand, from an unpublished DNA barcode submission to GenBank in 2010/2011 (see Appendix) and recent reports from there (e.g. Malliga 2010; Loongyaii *et al*. 2011). *Eudrilus* is used for soy bean residues and rice husks vermicomposting in Malaysia (e.g. Lim *et al.* 2011; Shak *et al.* 2014) with the worms apparently imported as cocoons from India. It is also reported from Indonesia where vermiculture operations in Solo, Central Java are advertised (e.g. Indonetwork 2014; Cacinglumbricus 2014). This has now been confirmed by the Animal Husbandry Faculty at Bogor Agricultural University, West Java (Andy Darmawan pers. comm. via email Nov. 2014). Recent reports from Vietnam are from the provinces of Lang Son and Cao Bang by the Research Institute for Aquaculture [The Anh *et al.* (2011); AFSPAN (2012) but mispelt "*Eudrilus euganaie*"].

New Zealand records by Beddard (1895: 149), repeated by Michaelsen (1900, 1903), Hutton (1904: 355), Gates (1972), and Sims and Easton (1985) were stated by Thompson (1922: 359), Benham (1950) and Lee (1959: 365) to be an error introduced

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when Beddard (1891, 1895: 149) somehow mistook for *Eudrilus eugeniae* Smith's 1886 report of *Endrilus* [sic lapsus for *Eudrilus*] *levis* [= *Octochaetus*? *levis* (Hutton, 1877)] from Taranaki. Recent personal surveys of vermicomposting operations in New Zealand also failed to locate this species there (e.g. Blakemore 2012*a*).

The claim from the French islands off the coast of Newfoundland (St Pierre and Miquelon) of *E. lacazii* by Perrier (1872) was disputed by Gates (1982: 72), although this is possibly Gates's mistake as its type locality is Martinique in the Antilles, where there is also a town named St Pierre, rather than the one near Miquelon. No records of cultivation are confirmed from Germany (the supposed source some worms in the Philippines and South Africa), from a few southeast Asian countries neighboring Vietnam, or yet from China/Taiwan.

Locality: Specimens were collected from worm farms in Brisbane (1991) and samples sent to the author from Mackay, Qld (1992), and Menai, NSW (1996) [now in CSIRO/ANIC, Canberra with registration nos. RB.95.9.4/11.2 (Blakemore 1995)]; also confirmed from lowland Philippines (specimens in Fishery collection of UPV, Miagao) but only close to worm beds; neither was it located ferally in surveys on Negros Island (pers. obs. 2009–2014, cf. Flores 2007).

Habitat: Originating in shaded savannahs of West Africa, it now thrives in worm beds on worm farms; it is reported in natural high moisture/organic sites such as waterfalls or riverbanks on Guadeloupe and also in gardens and some vegetable or fruit fields in South America (Brown & Fragoso 2007: 372). It is newly found in vermifilters of aquaponics tanks at Sulu Gardens in Miagao, Philippines (pers. obs. Feb. 2014).

Behaviour: Hatchlings are reported to sometimes return to the cocoon when alarmed. Active with a rapid escape response when disturbed, but if captured the adult worms become placid and can be readily handled. The species will wander at night, leaving plant pots and escaping unsealed containers when there is no light source. For rapid field identification, slight pressure between the fingers will cause eversion of white penes that are shaped similar to a scorpion's stinger (see Fig. 2).

DISCUSSION

Life cycle

The genus *Eudrilus* is bi-parental, being characterised by internal fertilisation preceding cocoon production (Sims 1964, 1987). Initial life studies are relatively recent, for example by Neuhauser *et al.* (1979), who found the best growth on horse manure or activated sewage sludge. Its life span can be 1–3 years, with *Eudrilus eugeniae* possessing a life cycle that ranges from 50–70 days, with sexual maturity reached at 35–50 days in culture (Viljoen & Reineke 1989). Reineke and Viljoen (1988) reported that in a cattle manure substrate at 25°C, cocoons produced by adult worms between the ages of 70–100 days were incubated for *ca.* 17 days before producing a mean of 2.7 hatchlings per cocoon (range 1–8) with 84% hatchling success. Dominguez *et al.* (2001) had similar findings with cocoons hatching in only 12 days at 25°C, and reaching maturity in as little as 35 days (total 47 days). The latter findings concluded that mature worms produced 3.6 cocoons per week with 2.2 viable hatchlings per cocoon (= 6.5 hatchlings per worm per week on average). Viljoen and Reinecke (1989, 1994) reported the first indication of clitella at between 35–45 days; worms with fully developed clitella copulated readily

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and the formation of cocoons started within 24 hours after copulation, continuing for up to 300 days. In India, Nagavallemma *et al.* (2004) recorded an 18-fold increase in population (from 55 specimens to 1,007) on legume leaf/cow dung substrate in three months, the highest of three composting species they tested. Also in India, Vasanthi *et al.* (2013*b*) report the highest productivity rates in sugarcane filter mud (=pressmud) mixed with sawdust and cattle manure at 26°C. These rates of growth and reproduction are amongst the highest currently reported for any earthworm.

Parasites and disease resistance

Gates (1982: 74) states that, unlike in most earthworm species, parasitic protozoans had not been reported. Internal nematodes (parasitic, paratenic or commensal) are known (e.g. Gates 1974: 74; Poinar 1978; del Valle & Rodriguez 1988; McNeill & Anderson 1990; Spiridonov 1992), but this species is supposedly disease-free apart from records of ammonia lesions on the clitellum (Gerasimov 2007). The functions of the ancillary glands of the female and male organs are not fully worked out; possibly they produce nutrients for eggs/sperm or are in part copulation sentinels preventing intromission of parasites or other disease vectors. Neither is the function of the supra-intestinal glands understood, as already noted.

Regeneration

Gates (1982: 74) reports 'head' regeneration as well as more easily observed posterior or 'tail' regeneration, and sometimes abnormalities like forked tails. Regrowth findings were confirmed, e.g. by Parida and Swain (2011) and Subashini *et al.* (2014), thus it is plausible for this species to get two viable worms from a single 'individual' as with some other species reported by Blakemore (2001).

Ecology and economics

Ecology of the three most common aerobic composting worm species, *Eisenia fetida* (Savigny, 1826), *Eudrilus eugeniae* and *Perionyx excavatus* Perrier, 1872, that are most often bred in worm farms and fed on household vegetable wastes or animal manures, are detailed in reports by Graff (1982), Sabine (1983), Kale and Bano (1991), Reinecke *et al.* (1992), Kale and Sunitha (1993, mispelt "Sunita" in Edwards 2004 : 388, table 19.3) and by Dominguez *et al.* (2001). Comparative studies generally show *E. eugeniae* to be a most productive species in tropical zones or under cover in temperate regions (where it is bred mainly for fish bait) since its large size makes it particularly easy to handle and harvest. As well as its use for fishing bait, this species is also used as a commercial meal high in protein for fish, birds and other animals that reject the taste or smell of *Eisenia fetida*, as noted below. For example, Gates (1982: 74) says it is the preferred food for duck-billed platypuses [*Ornithorhynchus anatinus* (Shaw, 1799)] in zoos, and this was confirmed by the Qld National Parks and Wildlife Service in Brisbane (pers. obs.).

In Cuba, India and the Philippines, this worm is favoured most for producing vermi-compost fertiliser for organic farming, whereas in North America and Australia the main commercial use is for breeding as fish bait where it is known colloquially as the 'African Nightcrawler' or 'ANC'. The current studies noted a propensity to escape from containers at night and wander, adding justification to this common name. Despite its potential for mobility, there were no records of natural colonisation for North America (Gates 1958, 1972, 1982) or Australia, and such records from New Zealand are now

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known to be mistaken identities. As Gates (1958: 10) said: "This species, originating in tropical Africa and until very recently known only from the tropics, has been raised and distributed in the United States for several years by earthworm culturists. Sales appear to be mostly to anglers for bait. Escapes of live specimens into natural environments must have been numerous. As yet, however, there are no records to indicate acclimatization and permanent colonization in mainland states." This was slightly counter-indicated by his later record of specimens from soil under oak trees at Vero Beach Laboratories, Florida (Gates 1982: 72).

Nevertheless, the spread of *Eudrilus eugeniae* may require closer monitoring as it is a widely cultivated species in Brazil too and there are some records of its survival away from worm farms, mainly in areas of high organic matter, but also in gardens and fields. It was also collected from waterfalls and river sites in Guadeloupe (Csuzdi & Pavlicek 2009). A novice report from mountainous forests in Negros Occidental, Philippines (Flores 2007) is unsubstantiated, being based on a simplistic, non-specialist key only to families, and re-surveys (unpub.) by the current author have not found it far from culture beds.

Preferring bedding material rich in organic matter in culture, this savannah worm also survives in unamended soils (M'ba 1978); and Blakemore (1994) successfully maintained it for six months with reproduction in mesocosms of heat-sterilised but unamended clays (vertisol and kraznozem) and sandy (podzol) soils in the glasshouse. Parthasarathi (2007) showed that over a year it will grow in clay loam but not as well as in composts where its biomass may be six times as high; thus it is considered adaptable to a wide range of soil types, unlike most other highly restricted earthworms that co-evolve with their soils (Michaelsen 1922).

Such environmental tolerance was investigated by way of soil selections by Madge (1969) who introduced *Eudrilus eugeniae* and another tropical species to gradients of soil texture and found a marked preference for the 0.25 mm particle size fraction (fine sand) over both coarse and very fine sand. A series of choice trials conducted by Blakemore (1994) showed that it, along with *Eisenia fetida*, had a tendency to select soil amended with manure when given a choice and compared to other species; it was found in clay soil rather than loam or sandy soil in 25% of all its observations. Habitat requirements were also tested by Madge (1969), who introduced *E. eugeniae* and another tropical African species to a soil moisture gradient and found 65% of the earthworms in the 12–17% moisture sectors after 48 hours; tolerated pH range was between 5.6–9.2 and for temperatures, he found an optimal range between 23°C and 31.5°C. In growth experiments, Viljoen and Reinecke (1992) reported that no *E. eugeniae* juveniles survived below 12°C or above 30°C, and optimal temperature for growth and reproduction was around 25°C. Attempts to establish it in natural environments show that the worms do well until the temperature drops to 40°F (4.4°C), at which time they die (Gates 1972: 52). Sims and Gerard (1985, 1999) say that temperatures below 10°C are not tolerated and that the optimum breeding temperature range is 21–27°C. Domingues *et al*. (2001) confirmed optimum moisture and temperature ranges for growth of near saturation at 80–82% and 25–30°C. These moisture and temperature levels correspond well to findings by Blakemore (1994), except the preferred moisture range was 20–25% in a light sandy loam (10% clay) and the preferred temperature was 25°C, in gradient cylinders laid horizontally to circumvent depth affects.

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Nature of Eudrilus *casts*

Eudrilus eugeniae is plentiful in the coastal, shaded savannah grasslands of its West African homeland where copious surface pellets are produced — remarkably, up to 140 t ha^{-1} per annum is deposited (Madge 1969) or equivalent to 156.8 t ha^{-1} per annum according to Gates (1972: 52), during the rainy season only.

In a series of mesocosm soil cores trials with combinations of species, soils and test crops, Blakemore (1994, 2008*a*) determined that *E. eugeniae* produces distinctive elongated pellet-like casts (2–3 mm × 1 mm) on the surface of the soil, especially near container edges where the worms burrowed, and had the highest surface cast production (equivalent to between 9.8–14.0 kg m^{-2}) of a dozen species tested, supporting the findings for a high casting rate of *E. eugeniae* estimated by Cook *et al.* (1980) of up to 2.43 g soil per gram fresh weight worm per day. The casting rates in the 23 cm diameter (0.0434 m^2) by 33 cm deep mesocosms were as high as 610.5 g per pot in kraznozem soil in just six months, equivalent to 281.3 t ha^{-1} per annum, if extrapolated to the field, or about twice the rate reported by Madge (1969).

Blakemore (1994, 2008*a*) observed that, where casts for this species were deposited on the surface, adventitious plant roots sought them out and that casts fallen over the lip of the cores into the base tray also had root hairs firmly attached to the pellets at the base too; when the roots were lifted cast pellets looked like miniature bunches of grapes dangling on a vine. This may be explained by *Eudrilus eugeniae* having significantly higher nutrients and trace elements in its casts compared to the clay soil matrix, especially nitrate-N, K and Zn, which were about twice that of the soil (Blakemore 1994, also Table 1), and possibly other plant attractant compounds.

Effects on plant yield and soil moisture after deliberate introduction of this species

Comparative glasshouse experiments and field trials (Blakemore 1994, 1997, 2008*a*) showed statistically significant yield increases when this worm was introduced to soil cores ranging from +21–74 % for oats and +15–50 % for grass shoots (and up to +50 % for grass roots), compared to uninoculated pots. Infiltrations rates were increased 19.0× in clay and 3.6× in sandy soil cores in the glasshouse, this partly attributed to absorption by surface casts and also drainage in worm burrows. As one of a dozen candidate species later tested in two field inoculation trials, *Eudrilus eugeniae* significantly increased pasture grass yield by 83.9 % (i.e. nearly doubled compared to controls). However, these preliminary results were considered inconclusive due to background variation and lack of survivors after one year during a particularly severe drought in tropical eastern Australia that precluded irrigation even from cattle stock reservoirs.

Inadvisability of deliberate introductions of alien species

The ethics of releasing an exotic species such as *Eudrilus eugeniae* into the Australian field were approved by PhD supervisors at CSIRO Tropical Agriculture, Qld, since this was an objective of the project that aimed to enhance improved pasture production naturally (Blakemore 1994). Possible risks were considered acceptable on the grounds that this species was present in Australia, having been legally imported from Canada by a worm grower some years previously; moreover, it was already widely sold for fishing bait, including in the Mundubbera township nearest to the release site (pers. obs.). This vermicomposting species is sexual (i.e. it requires mutual partners to reproduce rather than being parthenogenetic) and the upland release area, classed as dry, arid sub-tropical,

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TABLE 1

Composition of Eudrilus eugeniae casts produced from two types of unamended soils: (A) a sandy podzol and (B) a clay vertisol (Blakemore, 1994: tables 4.3.22/23) compared to (C) 100% ANC vermicast mainly from horse manure (data courtesy of Kahariam farms). Ratios in square braces are relative to the source soil medium; 1% = 10,000 mg/kg or 10,000 ppm (parts per million); '~' = conversion estimates.

Component	(A) Sand soil casts	(B) Clay soil casts	(C) 100% Vermicast/ compost
Moisture	-	-	30%
pH	6.2 [1.0]	7.0 [1.0]	6.8
Organic matter	~2.1% [0.9]	~8.5% [1.0]	36%
C:N Ratio	17:01 [0.9]	13:01 [0.9]	15:01
Total C	1.2% [0.9]	5.0% [1.0]	~21–28%
Total N*	0.07% [1.0]	0.40% [1.1]	1.89%
P2O5 (from P-Bicarb)*	0.005% [0.8]	0.049% [1.0]	2.49%
K2O (from K total)*	0.13meq% [0.7]	3.4meq% [1.9]	1.40%
NO3-N	3 [0.3]	44 ppm [1.9]	-
SO4-S	6ppm [0.7]	17ppm [1.3]	-
Ca	2.2meq% [0.8]	26meq% [1.0]	5.09%
Cu	0.5ppm [[0.8]	9.5ppm [0.8]	95ppm
Fe	-	-	2.63%
Mg	0.76meq% [0.8]	11meq% [1.0]	0.17%
Mn	35ppm [1.0]	77ppm [0.8]	1,233ppm
Zn	6ppm [0.8]	37ppm [1.9]	329ppm
MEAN ratio to soil	[0.8] or -20%	[1.2] or +20%	-

*Total macronutrients N-P-K are generally less important for composts that are microbially activated.

was several kilometres from a water course that may have aided dispersal. Thus the likelihood that the species would persist due to the single release event, especially in the prevailing drought, was considered negligible. However, increasing concerns about the spread of alien species would mean it is inadvisable to contemplate such a release or deliberate introduction in the future in Australia or elsewhere. Commercial solutions for the worm bait/compost market could be found from the pool of native species, some of which are already bred as bait in Australia (Blakemore 1999, 2012*b*) but, again, redistribution of these natives is also ill-advised albeit less objectionable and less regulated (by import/quarantine restrictions) than the use of exotics.

Microbial aspects

Sruthy *et al.* (2013) determined that the intestinal microbial populations of *Eudrilus eugeniae* in the foregut, mid and hindgut were dominated by bacteria, actinomycetes and fungi, respectively. These authors reviewed the diversity of types and number of these microbes, plus yeasts and protozoans in the casts of *E. eugeniae* and other vermicomposting species reported on by other researchers (see next section below). Use of earthworms in vermistabilisation of sewage sludge and other wastes have had

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favourable results when attempted in various regions (e.g. Neuhauser *et al.* 1988; Blakemore 2000*b*, *c*). Monroy *et al.* (2008) showed that processing of pig manure slurry with *E. eugeniae* eliminated nematodes and reduced coliform bacteria by up to 98%.

Composition of ANC vermicompost

The chemical and microbial characteristics of *E. eugeniae* vermicast/vermicompost differ depending upon the nature of the substrate and the age of the casts. Chemical composition is determined by the source material on which the worms are fed but is often enhanced in terms of natural plant nutrients, these changes relating to physical, chemical and microbial activities during and after passage through the worms' intestines. Table 1 summarises data from experiments by Blakemore (1994: tables 4.3.22, 4.3.23) that found that plant nutrients in casts of clay soil increased by about +20%, whereas casts from a sandy soil were depleted in nutrients (by -20%) probably due to assimilation by the worms, although structural characteristics of the soil matrix were improved by the worm activities too, increasing plant yields as noted above. Several authors, have confirmed phytohormone-like effects of casts as reported by Tomati *et al*. (1988). For instance, Nagavallemma *et al.* (2004) found generic vermicomposts to have higher percentages (nearly double) of both macro- and micronutrients and higher microbial activity compared with garden composts, plus they detected these plant-growth-promoting agents. Data from Parthasarathi (2006) is summarised in Table 2.

It is from the microbial activity that most of the benefits of worm casts accrue, relating to mineralisation and the gradual release of nutrients, as well as plant-growth-promoting enzymatic agents. As vermicompost ages, this biological activity declines, but the vitamin content may double with time (Prabha *et al.* 2007). Studies by Parthasarathi and Ranganathan (2000) showed that enzymes (cellulase, amylase, invertase, protease and phosphatase) declined as the casts aged. Parthasarathi (2006) found that *Eudrilus eugeniae* fed on sugar cane pressmud had a more than four-fold increase (significant $p<0.05$) in microbial population (fungi + bacteria + actinomycetes) and dehydrogenase enzyme activity in fresh casts, leading to enhanced nutrient mineralisation, but this activity gradually decreased over the period of a month as the casts aged (see Table 2).

Effects of ANC vermicompost on plant yield

A study by Kale *et al.* (1992) commented on applications of *Eudrilus eugeniae* vermicompost on crops: application equivalent to 5 t ha^{-1} plus half of the recommended chemical fertiliser gave a significantly higher yield of rice in outdoor pots. The rice results are similar to those reported by Pontillas *et al.* (2009) from the Philippines. However, the combination of vermicomposts with artificial fertilisers and other agri-chemicals may actually reduce efficacy, since there is often a need to adopt wholly organic methods, not just for organic certification, but also based on certain long-term studies, such as Blakemore (2000*a*) which found that a partly organic section of Haughley Farm was not as productive as a wholly organic section. Unpublished data from Kahariam organic farm in the Philippines show that ANC vermicast application to paddy at rates of just 1.5–3 t ha^{-1} are adequate to increase rice yields to 90–120 cavans ha^{-1}, well above the typical local range of 20–90 cavans ha^{-1} but without the need for chemical additions (Mr Danny Rubio, farm manager, pers. comm.). Similarly, an organic sugarcane fields on Negros applying up to 30 t ha^{-1} *Eudrilus* vermicompost yields 90 t ha^{-1} (Mr R. Peñalosa, pers. comm.), above the regional average yield of 50 t ha^{-1}, i.e. +80%. In current studies by

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TABLE 2
Microbes in ANC vermicompost (after Parthasarathi, 2006: table 1); CFU = Colony Forming Units.

Casts	Bacteria (CFU g-1)	Fungi (CFU g-1)	Actinomycetes (CFU g-1)
Fresh casts	$175{,}800 \times 10^4$	346×10^4	660×10^4
30-day-old casts	$101{,}800 \times 10^4$	215×10^4	280×10^4

the author, the resident earthworms on both farms were enhanced in terms of biomass and biodiversity when compared with neighbouring farms that use conventional chemical fertilisers and biocides. Such findings attest to the sustainability of organic farming using vermicomposts as primary fertilisers.

Composition of fish bait, stock feed and worm meal

Worms can be fed to stock directly, or dried and added to worm meal. Composition is provided by several authors, for example Hertrampf and Piedad-Pascual (2000) who show 85.3% moisture in *E. eugeniae* worms with 56% protein when dried, and ten essential amino acids plus macro and trace minerals of freeze-dried vermimeal. Although earthworm feeds tend to be a rich source of vitamins A, B and D, specific data for *E. eugenia* are not presently available.

Radioactivity and biocide effects

Eno (1966) found *E. eugeniae* to be less susceptible than *Lumbricus terrestris* Linnaeus, 1828 to irradiation in the range 16–64 kR. In Nigerian soils, this worm had much higher concentrations of DDT and its products, compared with the surrounding soil, and its production of surface casts virtually ceased in DDT-treated plots, which was considered a contributory factor to the overall decline in fertility of these plots (Cook *et al*. 1980). The concentration of heavy metals (Cu, Zn, Pb, Cd and Hg) in the tissue of *E. eugeniae* fed on municipal wastes accumulated beyond acceptable levels for protein-meal production (Graff 1982), although this trait could be utilised for soil bioremediation.

Pharmaceutical or cosmetics uses

Earthworm fibrinolytic enzymes are a group of serine proteases that are used as anti-coagulent or thrombolytic drugs to treat and prevent cardiac and cerebrovascular diseases in heart and stroke patients. In original 1982 Japanese patents (e.g. US4568545A) extracts were said to come from "*Lumbricus rebellus*" (sic), but Indian researchers (Sharma *et al.* 2011*a*) have since cloned and sequenced the fibrinolytic protease (Efp-0) gene from *E. eugeniae* as well as *Eisenia fetida*. The antimicrobial, antioxidant, anti-inflammatory and anticoagulant properties of *E. eugeniae* and its extracts have been variously studied (e.g. Mathur *et al.* 2010*a*; Packia Lekshmi *et al.* 2014). When *Eudrilus eugeniae* was dried and powdered, it produced antimicrobial responses of between 45–90% inhibition on agar plates when assayed against seven human pathogens (Anjana *et al.* 2013). Extracts from this worm showed antibacterial and antifungal properties that varied depending on the formulation (Mathur *et al.* 2010*b*). Preliminary studies by Shobha and Kale (2008) found indications of possible control of plant soil-borne fungal and bacterial pathogens using *E. eugeniae* exudates. These findings are supported by Vasanthi *et al.* (2013*a*) using a paste from this worm to inhibit the growth of resistant/recalcitrant human pathogens of bacteria such as *Staphylococcus aureus* Rosenbach, 1884 ('Golden

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staph') and of fungi such as *Candida albicans* (Robin, 1853) ('Thrush'), indicating its antimicrobial properties.

Emulating the works of Cooper *et al.* (2004*a*, *b*), but specifically using *E. eugeniae* as the source, Dinesh *et al.* (2013) report on the cytotoxic effect of coelomic fluid on cancer cell lines of human HeLa cells, colon cancer cells, WBC malignant tumour cells and brain tumour cells, with reduction by up to 33%. Such studies indicate novel use of this earthworm for treatment of cancers.

Azmi *et al.* (2014) recently found earthworm extracts, including those from *E. eugeniae*, to have anti-wrinkling properties as potential new 'anti-aging' agents.

CONCLUSION

Eudrilus eugeniae is a remarkably versatile vermicomposting species of the tropics or indoors in temperate regions. It is useful for recycling soil organic matter (i.e. maintaining humus), enhancing carbon sequestration, soil bioremediation, providing stock-feed protein, bioprospecting for pharmaceuticals/cosmetics or perhaps silk production (from the Y-shaped glands as reported by Eisen 1900: 141), and, trivially, as fish bait. Its geographical range and the applications of its products are rapidly expanding, with a summary of its vermiculture potential provided by Li *et al.* (2010). The field-identification method noted in relation to the male pores protruding under finger pressure, and the mtDNA barcode data (Appendix), should allow rapid identification of this economically and ecologically important species and confirm its separation from similar *Eudrilus pallidus* (Michaelsen 1892: 216) from Accra, Ghana [?syns. *E. buettneri* Michaelsen, 1892; *Eudrilus ifensis* Segun, 1978, but see Sims 1965: 304)] plus its subspecies *E. pallidus atakpamensis* Michaelsen, 1913 and *E. simplex* Michaelsen, 1913 from Togo. The importance of correct eco-taxonomic identification of each particular earthworm is re-emphasised along with routine use of DNA profiles. *Eudrilus eugeniae* is iconic with its many realised and potentially beneficial applications being researched, unlike the 7,000 other earthworm species currently described but mostly neglected, and these perhaps just a fraction of the total numbers in nature (Blakemore 2012*b*). Thus, despite its long study history from Darwin (1881), the highly practical and applied field of earthworm ecology still remains largely open and unexplored.

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APPENDIX
mtDNA COI barcode data (BLAST from http://blast.ncbi.nlm.nih.gov).

P14 Eu. eugeniae from Kahariam farm, Philippines 21st Nov., 2013 in 70% ISO-Ethanol. Coll. RB/Rowena Ocenar.

Sequence (584bp):

TAAGACAGCCGGGTGCTTTTCTAGGAAGAGACCAACTCTATAACACTATCGTTACAGCTCAT
GCTTTTCTAATAATCTTTTTCCTTGTAATGCCAGTTTTTATTGGCGGATTTGGAAATTGATTA
CTCCCACTAATACTGGGAGCGCCCGACATAGCATTCCCCCGACTAAATAATTTAAGATTTTG
ATTATTACCTCCTTCACTAATTCTCTTAGTTTCGTCAGCTGCAGTTGAAAAGGGTGCAGGTAC
AGGATGAACTGTTTACCCACCACTTGCAAGAAATCTTGCTCATGCGGGGCCCTCAGTAGACC
TAGCCATTTTCTCTCTTCATCTTGCAGGGGCATCATCTATTTTAGGGGCAATTAACTTTATTA
CAACAGTAATTAATATACGATGATCCGGTCTTCGATTAGAACGAATTCCACTATTTGTATGA
GCAGTAGTAATCACTGTAGTGCTACTTCTTCTATCACTTCCAGTCTTAGCGGGGGCAATCAC
AATACTTCTCACAGATCGTAATCTCAATACTTCATTCTTCGACCCAGCTGGGGGTGGAGATC
CAATTCTTTATCAACATTTATTC

megaBLAST result: Eudrilus eugeniae (KC122194.1) from India, Id. 584/584 (100%), i.e. perfect match; Eudrilus eugeniae (HM219171.2) from Thailand, Id. 564/584 (97%), i.e. some difference. GenBank: HM219171.2 origin in Thailand not stated in publication by Loongyai *et al.* (2011).

P15 Eu. eugeniae from Kahariam, Philippines 21st Nov. 2013 in 70% Ethyl-alcohol. RB/RO.

BLASTn result: P14 vs. P15 Id. 584/584 (100%).

Note: *Eudrilus eugeniae* was said to have been introduced from West Germany to both India (in the late 1970s according to Meenatchi *et al.*, 2009) and to Philippines (in the early 1980s) thus supporting the same species origins. However, both these statements need to be tempered since Stephenson (1923: 486) already recorded it in India from 1898, and local growers said other stocks of *E. eugeniae* were imported to Philippines from Australia. Sharma *et al*. 2011*b* investigated DNA markers in *E. eugeniae* finding two strains, and Meenatchi's study identified six distinct molecular strains in their Indian samples with highest and lowest genetic similarity index values of 1.0 to 0.40. However, neither of these studies tested the COI gene, cf. GenBank: KC122194.1 from India by Gupta *et al*. "Earthworm for production of compost from coconut leaf biomass" (unpublished), submitted in November 2012.

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