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Original article

# Two New Species of Japanese Earthworms (Annelida, Oligochaeta, Megadrilacea, Megascolecidae) Update Biodiversity on Okinawa and at Lake Biwa to ca. 30 Species

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**Abstract.** Two large native worms are an *Amynthas* from Kumejima, Okinawa and a *Metaphire* from Lake Biwa, Shiga-ken both supported by mtDNA COI barcodes, and two new species are described. New 2016 Okinawa exotic reports are of small American lumbricid *Bimastos parvus* (Eisen, 1874) and confirming of Asian megascolecid *Pithemera bicincta* (Perrier, 1875).

**Key words:** exotics, megadrile earthworm, mtDNA barcoding, natives, new species

## Introduction

In 2015 an interesting image of a luminescent earthworm from Kumejima Island, Okinawa was uploaded to a natural history photo website (by Nature photographer Shawn Miller – Fig. 1). The next year, in 2016, an opportunity arose for the survey of earthworms on Kumejima to try to find and identify this possibly novel species.

Kumejima " 久米島 " is a volcanic, sub-tropical island shaped like the silhouette head of an English cocker spaniel (Fig. 2). No earthworms were previously reported from this island whereas other Okinawan Islands have a limited history of reports since Kobayashi (1941) described ten species and Ohfuchi (1956, 1957a,b) reported ~24 with current totals of ~30 Ryukyu megadrile taxa (Easton, 1981; Blakemore, 2003, 2020a, b).

Furthermore, another unknown earthworm from Kinki Region, Honshu, Japan was found. Both the earthworms from Kumejima and from the Kinki Region are described as new species herein.

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## Materials and methods

Representative Kumejima specimens collected in February, 2016 were deposited in Lake Biwa Museum (LBM, Accession Nos. FY2015-11) with small tissue samples taken for mtDNA barcoding at molecular genetics laboratories using standard routines for DNA extraction, PCR amplification and COI gene Sanger sequencing (e.g., Blakemore *et al.* 2010). Other earthworms donated to LBM's collection were simultaneously inspected with a summary of the current Lake Biwa worm biodiversity provided in an Appendix.

## Results

Specimens deposited in Lake Biwa Museum (LBM) and Kanagawa Prefectural Museum (KPM) are described below.

### *Amynthas kume* Blakemore, sp. nov.

**Distribution**: Mt Aratake, Kumejima Island, Okinawa, Japan (26°18'38.3"N 126°47'15.0"E); also reported from Mt Daruma by S. Miller (RJB pers. comm.).

**Etymology**: The species name is derived from its island location (noun in apposition).

**Material examined** (see Figs. 3–4): Holotype (H), LBM FY2015-11-1 mature, dissected (DNA tissue

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Fig. 1. Mystery luminescent Kumejima Island earthworm (photo: Shawn Miller).

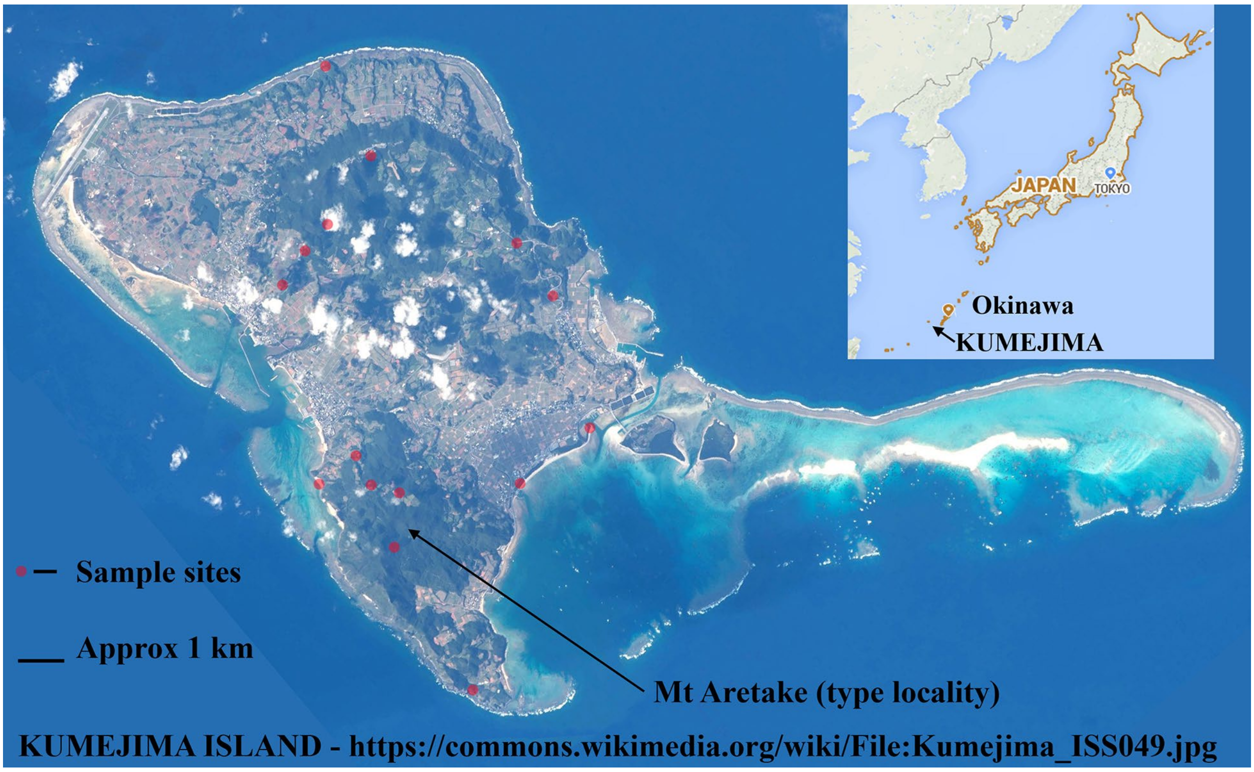


Fig. 2. Satellite image of Kumejima with collection sites (Wiki Commons: ISS049.jpg).

Fig. 3. *Amynthas kume* sp. nov. from Kumejima Island (photo courtesy of Shawn Miller).

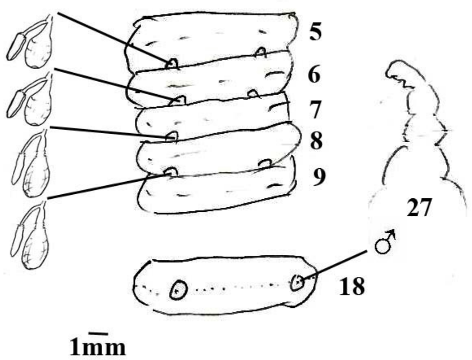


Fig. 4. *Amynthas kume* sp. nov. Holotype sketch with spermathecae and caecum.

samples #K3-5.1, #K5.2 re-sent to DNA lab via KPMNH); Paratype 1 (P1) FY2015-11-2 (DNA #K1); Paratypes 2–3 (P2–4) FY20015-11-3–4; all collected 15th Feb., 2016 (by RJB) from under forest litter or logs beside mountain road.

**Description**: Large and brown with yellow/buff intersegmental bands (i.e., striped). Lengths, H 190+20 mm (posterior amputee = 210 mm); P1, 220 mm; P2, 205 mm; P3, 190 mm. Prostomium epi-lobous. First dorsal pore 11/12. Setae about 60 on segment 12. Spermathecal pores in line with male pores four pairs in 5/6/7/8/9 in semi-circular pits. Clitellum 14–16. Female pore on 14. Male pores superficial in small circular patches on 18 with about 14 (H) or 12 (P1) setae intervening between pores. No genital markings (GMs) found. Septa 8/9/10 absent around muscular gizzard in 8. Spermathecae four pairs in 6–9 each with single diverticulum about as long as ampulla. Seminal vesicles in 11 and 12. Ovaries and last hearts in 13. Small ovisacs in 14. Intestine from 15. Racemose prostates in 18. Intestinal caeca smooth and simple in 27–24. No glands found near spermathecal pores.

**Diagnosis**: Other megascolecid species currently reported from Okinawan Islands with four pairs of spermathecae in segments 5/6/7/8/9 are exotic *Amythas corticis* (Kinberg, 1867) species-complex and *A. micronarius* (Goto & Hatai, 1898) parthenogenetic species-complex with *A. obtusus* (Ohfuchi, 1957) currently in its synonymy (see Blakemore, 2012a, b, 2020). All these taxa have genital markings (GMs). A recent taxon on Okinawa Island is *A. cucurbita* Azama & Ishizuka, 2018 (emend.) that, however, is smaller at <75 mm and also has markings around its male pores. Quadrithecate *Metaphire riukiuensis* (Ohfuchi, 1957) has seminal grooves in 17–19 amongst other differences while the *M. formosae* (Michaelsen, 1922) group is mainly from Taiwan and adjacent islands. The present specimens thus appear unique to Kumejima representing a new species. It is unusual for an *Amynthas* to lack GMs, a trait more common to *Metaphire* that typically has fewer spermathecae and eversible penes to compensate.

Other earthworms found on the island in this study were the exotic lumbricid of American origin: *Bimastos parvus* (Eisen, 1874) (LBM FY2015-11-5) from Gushikawa Castle, Kumejima collected 16th Feb., 2016 from under rocks as a new record for Okinawa (published in Blakemore, 2016a), and Asiatic exotic *Pithemera bicincta* (Perrier, 1875) (LBM FY2015-11–6 providing tissue samples #K2 & K6 – KPM-NJL 82) that had been previously reported from Okinawa, not least by Ohfuchi (1957a: 254), Easton (1981) and Blakemore (2002, 2003, 2020a,b; Blakemore *et al.*, 2007). Despite Ito *et al.*'s (2019) claim, Ohfuchi's description precisely matches *P. bicincta* and he clearly states: "*Intestine begins in XIV, and a pair of intestinal caeca which are very short, are less developed compared with many other species; broad, apparently rudimentary, or may be altogether absent..*" but he has then inadvertently added a transcription error with: "*..commences (sic) in XXVI or XXVII..*" (in actuality its small caeca are often paired in 22). As for *B. parvus*, it is relatively common on the main islands of Japan and Kobayashi (1941) reports it from "海士村" that is translated as Ama mura, possibly on Nakanoshima in Oki Islands, Shimane-ken, but it has not been previously found on Okinawan Islands.

### *Metaphire kinki* Blakemore, sp. nov.

**Distribution**: Kinki Region, Shiga-ken, Otsu-shi, Katsuragawa, Boumura-cho (35.247719, 135.868247) and stream at Sakamoto just below the Mt. Hiei cable-car station (35.069342, 135.865797); Kyoto-fu, around Mt Kumotori/Kibune River (35°10'25.7"N 135°46'05.2"E) (Wes Lang pers. comm.: found "coiled up in the middle of the hiking path" from the bus stop at Hanase Kogen). Searches by RJB assisted by Dr Grygier in rivers and on hills at Sakamoto in Feb., 2016 failed to find further specimens.

**Etymology**: The name is derived from its regional location (noun in apposition).

**Material examined** (see Figs. 5–7): Holotype (H), LBM FY2015-8-1 mature, dissected (DNA tissue samples #K7–9, sample #K8 now KPM-NJL 83) collected by Kazuhiro Masunaga "*2015/8/1*"; "*On road where water runs across. Captured alive but died overnight. 80–85 % EtOH*"; Paratype 1 (P1) FY2015-8-2 large, macerated specimen (no DNA) ditto "*2015-8-2*"; Paratype 2 (P2) FY2010-24 "*Sakamoto coll. Masunaga*" March, 2011; Paratype 3 (P3) FY2011-19 ditto, "25.III.2012".

**Description**: Large and dark blue with light grey intersegmental bands (i.e., striped). Lengths, H 260 mm


Fig. 5. *Metaphire kinki* sp. nov. P2 specimen (photo courtesy Kazuhiro Masunaga March, 2011 with LBM permission via M.J. Grygier pers. comm. 22nd July, 2015).

Fig. 6. "Kumotori/Kibune" worm, likely *M. kinki* in Nature (image permissions courtesy Wes Lang https://tozantales.wordpress.com). Compare with uniformly-coloured, bright electric-blue *M. sieboldi* from Fukuoka and Kumamoto, Kyushu www.flickr.com/photos/pokoroto/2950708988; www.flickr.com/photos/anaguma/7540289876.

x 15 mm wide; P2, 210 mm; P3, 195 mm. Segments (H) ~140. Prostomium epi-lobous, open. First dorsal pore 12/13. Setae about 80 on segment 12. Spermathecal pores in line with male pores three pairs in 6/7/8/9. Clitellum 14-16. Female pore on 14. Male pores slits on 18 with about 16 (H) setae intervening. No genital markings (GMs) found. Septa 8/9/10 absent around muscular gizzard in 8. Spermathecae three pairs in 7-9 with single, coiled diverticulum longer than ampulla when unravelled. Seminal vesicles in 11 and 12. Ovaries and last hearts in 13. Racemose prostates in 18. Intestinal caeca from 27, deeply incised on one side at the base (not strictly manicate, more like intermediate or multiple, as found in *M. megascolidioides* – see Blakemore (2016a: Fig. 3 and Supplementary Materials, Appendix 1: Fig. 12).

**Diagnosis**: The only known native *Metaphire* species currently from Shiga-ken with three pairs of spermathecae in segments 6/7/8/9 is reported as "*M. sieboldi*" *sensu lato* by Minamiya *et al.* (2009). Major differences from the original and most subsequent species diagnoses of proper *M. sieboldi* (Horst, 1883) – the first species described from Japan – are lack of bright and uniform blue iridescent colouration and less defined manicate caecae; also the spermathecal diverticula may be longer than the ampullae even when they are coiled. The most definitive difference

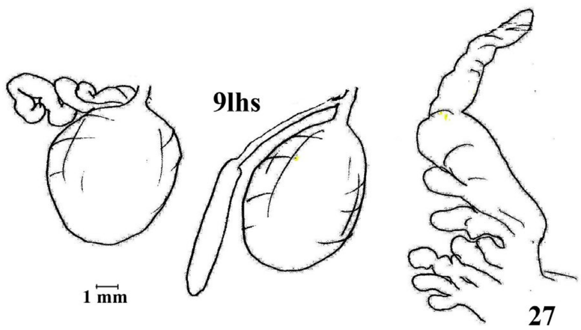


Fig. 7. *Metaphire kinki* sp. nov. holotype spermatheca 9lhs and caecum.

is the mtDNA COI barcode (in Appendix 1) that differs by 4.13 % from its closes match of AB482078 "*Metaphire sieboldi*" from Nara-ken that was part of Minamiya *et al.*'s (2009) "Group I" mostly confined to the Kinki region and also most diverged of their study samples. Only their AB482080, also Group I, was from Shiga-ken and it differs by 4.57 %. These authors' found: "*Among the* M. sieboldi *samples, the infraspecific sequence divergences of the COI gene ranged from 0.1 % to 18.1 %, and the mean infraspecific sequence divergences between phylogenetic groups of our study ranged 5.7 % to 15.9 %* (Table 2)."

However, an accepted COI barcode range for inter-specific differentiation is as low as 2–3 % (e.g. Hebert *et al.* 2003, 2004; Smith *et al.* 2005), also by Zhang & Zhang (2014) and by Wang *et al.* (2018) who found: "*almost 98 % of the species can be correctly distinguished for both COI and 16S when a threshold of 3 % nucleotide divergence was used for species discrimination*". As the current specimen divergence is >4 % from other known samples it would qualify, on the information currently available, for specific status. Its furthest BLASTn result differs from other sampled concepts of "*M. sieboldi*" by up to 14 % (see Appendix 1).

Other examples in the Appendix support an inter-specific COI gene division at >3–4 %.

*Metaphire sieboldi* proper is defined by its Leiden type (RNHL 1825) and any substantial derivation or deviation from this (e.g. DNA >3–4 %) warrants separate specific, or at least sub-specific status, seemingly as do most of Minamiya *et al.*'s (2009) group taxa.

Three LBM collection worms from Hokkaido dated "2014 09 11" sent by Y. Minamiya were identified (by RJB in 2016) as common exotic *Aporrectodea rosea* (Savigny, 1826).

About 35 species occur on the Ryukyu (Okinawan) Islands and all 30+ earthworm species reports from Lake Biwako's satoyama habitats are summarized in Appendix 1, several supported by mtDNA. Along with ~50 aquatic worm taxa, Biwako is Japan's most megadrile & microdrile biodiverse site due to intensity of eco-taxonomic survey.

## Discussion

Application of DNA to nomenclatural typification helps reduce taxonomic impediments. The first genetic analysis of worms (megadriles and microdriles) was by Siddall *et al.* (2001) and explicit linking of a genetic barcode to a worm's type was by Blakemore *et al.* (2010). Henceforth, molecular confirmations are advised for any eco-taxonomic surveys, albeit determining specific cut-offs between intraspecific and interspecific series, within *Amynthas* and *Metaphire*, as with other genera, is often difficult when a morphological continuum occurs. Yet DNA data are reliable only if initial identifications are good. Appendix 1 has several examples of misidentifications/misnomers relating to DNA data.

A suggestion for Genbank's DNA database is thus for a colour confidence system (or a kind of taxonomic triage) that, as with taxonomy, requires regular review, e.g.:-

RED – definitive, based upon taxon's primary types.

ORANGE – reliable, based upon taxon's secondary types.

GREEN – verified, on authoritative taxonomic identification.

BLACK – speculative or non-specialist identification of specimen.

[GREY or in braces – obvious misidentifications or highly unlikely taxon].

An unfortunate autocorrect feature of GenBank results in uploaded species names being changed according to some "official" list, not infrequently wrong, and also a modification of some uploaded sequences to comply with their models. Both these kinds of "corrections" detract from the validity of the unadulterated original data.

Returning to a supposed lack of molecular monophyly of both *Amynthas* and *Metaphire* (e.g., Zhang *et al.*, 2016), this is irrelevant to ICZN (1999) nomenclatural taxonomy where the genus is defined by characteristics of its type-species, as was clearly and simply explained by Blakemore (2002, 2008a, 2012c, 2020b). Thus male pores (when present!) are superficial in species defaulting to *Amynthas* and non-superficial in *Metaphire* species as, indeed, are found in its type *M. javanica* (Kinberg, 1867) [and its possible synonym, *M. californica* (Kinberg, 1867)]. Whereas the male pores in proper *Pheretima* type (*P. montana* Kinberg, 1867) are in deep invaginations, these are most developed as doubled and eversible penes in *Duplodicodrilus* [type *D. schmardae* (Horst, 1883) itself often misidentified as *Metaphire javanica* or *M. californica* in overseas reports (RJB pers. obs.)]. There is no implication that form of male pores is the same in these latter genera, merely that they are all "non-superficial".

Consequently, the recently described *Metaphire glauca* (Azama & Ishizuka, 2018) n. comb. (as mooted in Blakemore 2020a) from northern Okinawa complies with a *Metaphire houlleti* (Perrier, 1872) species-complex as in Blakemore (2016b, 2020b) that has >50 member taxa including many synonyms or parthenogenetic morphs that now need comparison with it. Several other taxonomic names proposed by these authors are possibly invalidated by non-compliance with ICZN (1999: Arts. 11.4 and/ or 11.5) by synonymy and homonymy (as noted by Blakemore, 2003, 2010, 2020a) (see Appendix 2).

Aside from classic ICZN nomenclatural taxonomy based upon morphology, now molecular DNA information plus behaviour and ecology are obviously needed to fully define a species. The new species *M. kinki* seemingly occurs often in streams or wet situations that may indicate an habitual characteristic separate from its sibling taxa.

Blakemore (2002, 2020b) speculated that bright blue colouration (only manifest in adults) of *Metaphire sieboldi*

Fig. 8. Large unknown worm from North Vietnam on the Phan Si Pan Mountain in Lao Cai district with striking blue-striped colouration (photo taken in 2016 courtesy of Dr Richard Barnes of Logan Botanic Gardens, Scotland, UK; pers. comm. April, 2018).

[as with *Didymogaster sylvatica* Fletcher, 1886 in NSW and *Diporochaeta terraereginae* (Fletcher, 1890) in tropical forests in Qld., Australia], may serve to distract predators, especially birds. Similarly for banding as in the two new taxa herein seen also in *A. yambaruensis* (Ishizuka & Azama, 2000) in Okinawa; *Fletcherodrilus fasciatus* (Fletcher, 1890) in Australia and *Eophila tellinii* (Rosa, 1888) or *E. crodabepis* Paoletti 2016 in Italy. Both characteristics are manifest in an unidentified megadrile found wandering on the soil surface in North Vietnam (Fig. 8).

During the Kumejima survey an abundance of several other native and exotic earthworms (plus enchytraids and planarians) were found in the woodlands and public areas, most not collected, but, remarkably, no earthworms were detected in the adjacent sugar cane fields. Possibly the earthworm fauna has been eradicated by intensive agrichemical use, a situation recently reported as a global issue of concern (Blakemore, 2018). This latter publication determined that organic sugar production offered substantial benefits in terms of yield, soil health (e.g. earthworm counts) and soil carbon sequestration compared to detriments of conventional agrichemical agriculture.

Other Kinki/Shiga species in LBM collection were described in Blakemore & Kupriyanova (2010), Blakemore (2010, 2016a) and Blakemore & Grygier (2011) that, with the current new species, now bring known Lake Biwa region taxa to about 30 megadriles, the most biodiverse habitat currently in Japan (see summary and DNA data in Appendix 1). This earthworm biodiversity compares to ~21 megadrile taxa from Lake Pedder in Tasmania (Blakemore, 2010: tab. 2) and other examples from Blakemore (2018) such as: "*a mixed farm in Queensland, Australia had 24 earthworm species whilst an organic farm studied in the Philippines had 23 [17,47,48]. In one instance, 13 species were unearthed from gardens at Y Plas House at Machynlleth, Wales in half-a-day's collection in 2013 [49] matching the earthworm diversity reported at another UK site sampled for an entire 10-year period (1990–2000) [50].*" This Y Plas site report (Blakemore, 2013) was the most biodiversity in UK or Ireland until surveys found 19 lumbricid earthworm species at Isabella Tree's and Charlie Burrell's Knepp Castle rewilding farm in England (Cole, 2013). Such high counts from proper eco-taxonomic surveys contradict hackneyed claims that earthworms have but few "common" species per site.

The present report brings the number of known natives to about 82 plus more than 42 exotics in Japan (including new records of *Dendrobaena veneta* and *Lumbricus terrestris* as described in this Bulletin issue) to give a total tally of ~124 earthworm species (Easton, 1981; Blakemore, 2003, 2012d, 2019a, 2020a cf. Minamiya, 2021). However, full taxonomic review is yet required as classical Japanese earthworm types are unearthed after their removal and hiding, as discovered and reported by Blakemore & Ueshima (2011) and Blakemore (2012b, 2016a: Figs. 1–2) (see Appendix 2).

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## 摘　要

Blakemore, R. J., S. Miller & S. Y. Lim, 2022. 日本産フトミミズ科の 2 新種（環形動物門，貧毛綱，Megadrilacea）. 神奈川県立博物館研究報告（自然科学）, (51): 95–104. [Blakemore, R. J., S. Miller & S. Y. Lim, 2022. Two New Species of Japanese Earthworms (Annelida, Oligochaeta, Megadrilacea, Megascolecidae) Update Biodiversity on Okinawa and at Lake Biwa to ca. 30 Species. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci*), (51): 95–104.]

形態学的特徴および mtDNA の COI バーコーディングに基づき、沖縄県久米島産のアズマフトミミズ属の新種と、滋賀県琵琶湖産のフクロフトミミズ属の新種を記載した。また、2016 年に採集された標本に基づき、アメリカ原産のフクロナシツリミミズ *Bimastos parvus* (Eisen, 1874) と東南アジア原産のフィリピンミミズ *Pithemera bicincta* (Perrier, 1875) を沖縄県からの初記録として報告した。

**Supplementary Materials**

**Appendix 1.** DNA analyses support IDs with earthworm biodiversity table at Lake Biwa compared to Samford, Qld. (plus some items of taxonomic “housekeeping”).

Supplementary file Online. Available from internet: https://archive.org/details/appendix-1

**Appendix 2.** On rediscovering some of Japan’s historical & heritage earthworm types (with confirmed *Eisenia japonica* record in Japan from 1876; also an earlier *Amynthas vittatus* or “*Metaphire levis*” USA record here corrected as *A. tokioensis*). Thus the Odawara earthworm, *Amynthas vittatus* (Goto & Hatai, 1898) (Appendix 2: Fig. 16), is not proven to occur in USA and its tentative report from there should be scratched in favour of *A. tokioensis* (Beddard,1892) that already has many USA records (Appendix 2: Fig. 17).

Supplementary file Online. Available from internet: https://archive.org/details/appendix-2